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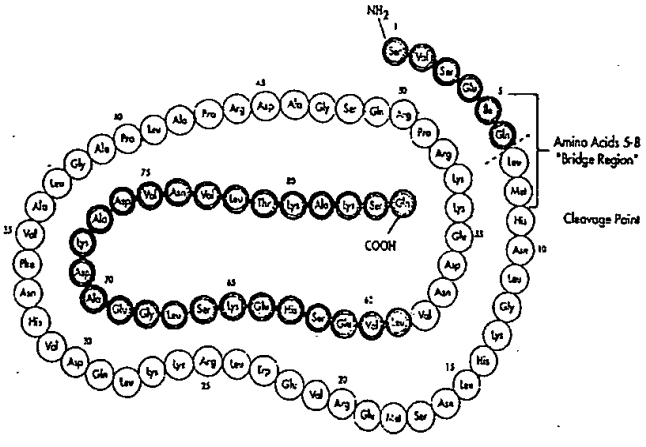
(54) Title: METHODS, KITS AND ANTIBODIES FOR DETECTING PARATHYROID HORMONE



(57) Abstract: The present invention relates to novel methods and compositions useful for detecting whole parathyroid hormone at a physiological level and parathyroid fragments in a mammalian sample. Such detections may be useful to different diseases or disorders in a subject, such as hyperparathyroidism and related bone diseases, from normal or non-disease states. One detects whole or non-fragmented (1 to 84) parathyroid hormone in a biological sample and optionally one or more of a selection of non-whole parathyroid hormone peptide fragments that may or may not function as a parathyroid hormone antagonists. By either comparing values or using independently the value of either the one or more of a selection of non-whole parathyroid hormone peptide fragments, the whole parathyroid hormone, or the combination of these values is able to differentiate parathyroid hormone and bone related disease states, as well as differentiate such states from normal states.

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METHODS, KITS AND ANTIBODIES FOR DETECTING PARATHYROID HORMONE

I. CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] The present application is a continuation-in-part of U.S. Patent Application No. 09/344,639, filed on June 26, 1999, now allowed; which is a continuation-in-part of U.S. Patent Application No. 09/231,422, filed on January 14, 1999, now allowed.

II. TECHNICAL FIELD

[0002] The present invention relates to novel compositions, methods and kits for differentiating parathyroid diseases in a subject. These compositions, methods and kits can be used, for example to differentiate hyperparathyroidism, high bone turnover, and adynamic bone disease from normal or non-disease states.

III. BACKGROUND OF THE INVENTION

[0003] Calcium plays an indispensable role in cell permeability, the formation of bones and teeth, blood coagulation, transmission of nerve impulse, and normal muscle contraction. The concentration of calcium ions in the blood is, along with calcitrol and calcitonin, regulated mainly by parathyroid hormone (PTH). Although calcium intake and excretion may vary, PTH serves through a feedback mechanism to maintain a steady concentration of calcium in cells and surrounding fluids. When serum calcium lowers, the parathyroid glands secrete PTH, affecting the release of stored calcium. When serum calcium increases, stored calcium release is retarded through lowered secretions of PTH.

[0004] The complete form of human PTH, (hPTH), is a unique 84 amino acid peptide (SEQ ID NO: 1), as is shown in FIGURE 1. Researchers have found that this peptide has an anabolic effect on bone that involves a domain for protein kinase C activation (amino acid residues 28 to 34) as well as a domain for adenylate cyclase activation (amino acid residues 1 to 7). However, various catabolic forms of clipped or fragmented PTH peptides also are found in circulation, most likely formed by intraglandular or peripheral metabolism. For example, whole PTH can be cleaved between amino acids 34 and 35 to produce a (1-34) PTH N-terminal fragment and a (35-84) PTH C-terminal fragment. Likewise, clipping can occur between either amino acids 36 and 37 or 37 and 38. Recently, a large PTH fragment referred to as "non-(1-84) PTH" has been disclosed which is clipped closer to the N-terminal end of PTH. (*See* LePage, R., *et al.*, *Clin. Chem.* 44: 805-810 (1998).

[0005] The clinical need for accurate measurement of PTH is well demonstrated. Serum PTH level is one of the most important index for patients with the following diseases:

familial hypocalciuric hypercalcemia; multiple endocrine neoplasia types I and II; osteoporosis; Paget's bone disease; primary hyperparathyroidism - caused by primary hyperplasia or adenoma of the parathyroid glands; pseudohypoparathyroidism; and renal failure, which can cause secondary hyperparathyroidism.

[0006] PTH plays a role in the course of disease in a patient with chronic renal failure. Renal osteodystrophy (RO) is a complex skeletal disease comprising osteitis fibrosa cystica (caused by PTH excess), osteomalacia -unmineralized bone matrix (caused by vitamin D deficiency), extraskeletal calcification/ossification (caused by abnormal calcium and phosphorus metabolism), and adynamic bone disease (contributed to by PTH suppression). Chronic renal failure patients can develop RO. Failing kidneys increase serum phosphorus (hyperphosphoremia) and decrease 1,25-dihydroxyvitamin D (1,25-D) production by the kidney. The former results in secondary hyperparathyroidism from decreased gastrointestinal calcium absorption and osteitis fibrosa cystica from increased PTH in response to an increase in serum phosphorus. The later causes hypocalcemia and osteomalacia. With the onset of secondary hyperparathyroidism, the parathyroid gland becomes less responsive to its hormonal regulators because of decreased expression of its calcium and vitamin D receptors. Serum calcium drops. RO can lead to digital gangrene, bone pain, bone fractures, and muscle weakness.

[0007] Determining circulating biologically active PTH levels in humans has been challenging. One major problem is that PTH is found at low levels, normally 10 pg/mL to 40 pg/mL (*i.e.*, 1 pmol/L to 4 pmol/L). Coupled with extremely low circulating levels is the problem of the heterogeneity of PTH and its many circulating fragments. In many cases, immunoassays have faced substantial and significant interference from circulating PTH fragments. For example, some commercially available PTH kits have almost 100% cross-reactivity with the non-(1-84) PTH fragment. *See* the LePage article *supra*.

[0008] PTH immunoassays have varied over the years. One early approach is a double antibody precipitation immunoassay found in U.S. Patent No. 4,369,138, issued to Arnold W. Lindall *et alia*. A first antibody has a high affinity for a (65-84) PTH fragment. A radioactive labeled (65-84) PTH peptide is added to the sample with the first antibody to compete for the unlabeled peptide. A second antibody is added which binds to any first antibody and radioactive labeled PTH fragment complex, thereby forming a precipitate. Both precipitate and supernatant can be measured for radioactive activity, and PTH levels can be calculated therefrom.

[0009] In an effort to overcome PTH fragment interference, immunoradiometric two-site assays for intact PTH (I-PTH) have been introduced, such as Allegro® Intact PTH assay by

the Nichols Institute of San Juan Capistrano, California. In one version, a capture antibody specifically binds to the C-terminal portion of hPTH while a labeled antibody specifically binds to the N-terminal portion of the captured hPTH. In another, two monoclonal antibodies were used, both of which attached to the N-terminal portion of hPTH. (For the purposes of the present disclosure, the complete form of human PTH is referred to as "whole PTH" or "wPTH" as distinguished from "intact PTH" or "I-PTH" which can include not only wPTH, but also a large PTH fragment cleaved about amino acids 5 to 8.) Unfortunately, these assays have problems in that they measure but do not discriminate between w-PTH and I-PTH. This inability comes to the fore in hyperparathyroid patients and renal failure patients who have significant endogenous concentrations of large, non-whole PTH fragments.

[0010] Recently, researchers have made a specific binding assay directed to the large N-terminal PTH fragments. *See* Gao, P., *et al., Clinica Chimica Acta* 245: 39-59 (1996). This immunochemiluminometric assay uses two monoclonal antibodies to detect N-terminal (1-34) PTH fragments but not mid-portion PTH fragments or C-terminal PTH fragments. A key factor in the design of these assays is to eliminate any reaction with C-terminal PTH fragments.

[0011] Nevertheless, specific whole PTH assays have not been able to measure whole PTH at physiological levels. *See, e.g.*, Magerlein, M., *et al., Drug Res.* 48:197-204 (1998). The present invention is intended to meet these and other needs in the art.

[0012] An important discovery leading to the present invention is that adynamic bone loses its capacity to buffer calcium and phosphate as the bones are shut down. In subjects afflicted with such conditions, they are unable to effectively buffer calcium as it enters their bodies through their diet. This calcium enters the blood stream and is thereafter shuttled to the soft tissues. The parathyroid gland is particularly subject to, and detrimentally affected by, this influx of calcium and thereby produces PTH fragments rather than, or in addition to, the active form of PTH. Accordingly, in subjects with adynamic bone, the concentration and production of PTH fragments is increased. In light of this and other related information, the measurement of PTH fragment levels, and particularly in conjunction with the measurement of whole PTH, can be used effectively to differentiate subjects having adynamic bone versus those having normal bone and high bone turnover rates.

[0013] There is a tremendous need to be able to non invasively separate the dialysis patients with ADN from those suffering from high bone turnover to avoid over treatment of ADN dialysis patients. Over treatment of dialysis patients with ADN is a frequent occurrence under presently utilized methods. For example, package inserts that proscribe the use of Zemplar® and Calcijex® (Abbott Laboratories), for example, are being used to treat thousands of dialysis patients that stand a great risk of over treatment under the proscribed protocols that

do not account for circulating total PTH fragment levels. The present invention addresses these and other need in the art.

IV. DISCLOSURE OF THE INVENTION

[0014] In one embodiment, the present disclosure provides an isolated antibody that specifically binds to an N-terminal sequence of whole parathyroid hormone (PTH) and is capable of detecting said whole PTH at a physiological level in a mammalian sample, with a proviso that said isolated antibody avoids binding to a non-whole PTH fragment. Frequently, the isolated antibody is a monoclonal or polyclonal antibody. Also frequently, the binding between the antibody and the N-terminal sequence of whole PTH is dependent on the presence of amino acid residues 2-5 or 3-6 of the PTH.

[0015] In one aspect an isolated antibody of the present disclosure specifically binds to an epitope comprised in PTH_{1-6}, PTH_{1-8}, PTH_{1-9}, PTH_{1-12}, or PTH_{3-12}. Frequently, an isolated antibody of the present disclosure specifically binds to the parathyroid hormone peptide PTH_{1-15} or PTH_{1-8}, wherein at least four amino acids in said peptide sequence are part of a reactive portion with the antibody. On occasion, an isolated antibody of the present disclosure specifically binds to an epitope comprised in PTH_{1-5}, PTH_{1-7}, PTH_{1-8}, PTH_{1-10}, PTH_{1-11}, PTH_{1-13}, PTH_{1-14}, PTH_{1-15}, PTH_{1-16}, PTH_{1-17}, PTH_{1-18}, PTH_{1-19}, PTH_{1-20}, PTH_{1-21}, PTH_{1-22}, PTH_{1-23}, PTH_{1-24}, PTH_{1-25}, PTH_{1-26}, $hPTH_{1-27}$, PTH_{1-28}, PTH_{1-29}, PTH_{1-30}, PTH_{1-31}, PTH_{1-32}, PTH_{1-33}, PTH_{1-34}, PTH_{1-35}, PTH_{1-36}, PTH_{1-37}, PTH_{2-5}, PTH_{2-6}, PTH_{2-7}, PTH_{2-8}, PTH_{2-9}, PTH_{2-10}, PTH_{2-11}, PTH_{2-12}, PTH_{2-13}, PTH_{2-14}, PTH_{2-15}, PTH_{2-16}, PTH_{2-17}, PTH_{2-18}, PTH_{2-19}, PTH_{2-20}, PTH_{2-21}, PTH_{2-22}, PTH_{2-23}, PTH_{2-24}, PTH_{2-25}, PTH_{2-26}, PTH_{2-27}, PTH_{2-28}, PTH_{2-29}, PTH_{2-30}, PTH_{2-31}, PTH_{2-32}, PTH_{2-33}, PTH_{2-34}, PTH_{2-35}, PTH_{2-36}, PTH_{2-37}, PTH_{3-6}, PTH_{3-7}, PTH_{3-8}, PTH_{3-9}, PTH_{3-10}, PTH_{3-11}, PTH_{3-13}, PTH_{3-14}, PTH_{3-15}, PTH_{3-16}, PTH_{3-17}, PTH_{3-18}, PTH_{3-19}, PTH_{3-20}, PTH_{3-21}, PTH_{3-22}, PTH_{3-23}, PTH_{3-24}, PTH_{3-25}, PTH_{3-26}, PTH_{3-27}, PTH_{3-28}, PTH_{3-29}, PTH_{3-30}, PTH_{3-31}, PTH_{3-32}, PTH_{3-33}, PTH_{3-34}, PTH_{3-35}, PTH_{3-36}, PTH_{3-37}, PTH_{4-7}, PTH_{4-8}, PTH_{4-9}, PTH_{4-10}, PTH_{4-11}, PTH_{4-12}, PTH_{4-13}, PTH_{4-14}, PTH_{4-15}, PTH_{4-16}, PTH_{4-17}, PTH_{4-18}, PTH_{4-19}, PTH_{4-20}, PTH_{4-21}, PTH_{4-22}, PTH_{4-23}, PTH_{4-24}, PTH_{4-25}, PTH_{4-26}, PTH_{4-27}, PTH_{4-28}, PTH_{4-29}, PTH_{4-30}, PTH_{4-31}, PTH_{4-32}, PTH_{4-33}, PTH_{4-34}, PTH_{4-35}, PTH_{4-36}, PTH_{4-37}, PTH_{5-8}, PTH_{5-9}, PTH_{5-10}, PTH_{5-11}, PTH_{5-12}, PTH_{5-13}, PTH_{5-14}, PTH_{5-15}, PTH_{5-16}, PTH_{5-17}, PTH_{5-18}, PTH_{5-19}, PTH_{5-20}, PTH_{5-21}, PTH_{5-22}, PTH_{5-23}, PTH_{5-24}, PTH_{5-25}, PTH_{5-26}, PTH_{5-27}, PTH_{5-28}, PTH_{5-29}, PTH_{5-30}, PTH_{5-31}, PTH_{5-32}, PTH_{5-33}, PTH_{5-34}, PTH_{5-35}, PTH_{5-36}, or PTH_{5-37}. Frequently, however, the non-whole PTH fragment is a peptide having an amino acid sequence from between PTH_{3-84} and PTH_{34-84}.

[0016] In a further embodiment a multiple antigenic peptide (MAP) is provided, which MAP comprises a branched oligolysine core conjugated with a plurality of a PTH peptide as described herein. On occasion, the branched oligolysine core comprises 3, 7 or 15

lysine residues, also on occasion, the MAP comprises 4, 8 or 16 copies of the PTH peptide. The plurality of the PTH peptide comprises the same or different PTH peptides. In one aspect, the plurality of the PTH peptide is conjugated to the branched oligolysine core via a spacer. Frequently, the spacer is an amino acid residue. Multiple antigenic peptides comprise generally known technology. *See, e.g.*, Adermann, K., et al., Innovations and Perspectives in Solid Phase Synthesis 429-32 (R. Epton, ed., Mayflower Worldwide 1994).

[0017] In another embodiment, the present disclosure provides a method for measuring a physiological level of whole parathyroid hormone in a mammalian sample, which method comprises: a) obtaining a sample from a mammal to be tested; b) contacting said sample with an isolated antibody that specifically binds to an N-terminal sequence of whole PTH and is capable of detecting said whole PTH at a physiological level in said mammalian sample, with a proviso that said isolated antibody avoids binding to a non-whole PTH fragment; and c) assessing a complex formed between said whole parathyroid hormone, if present in said sample, and said antibody, to measure physiological level of said whole parathyroid hormone in said mammalian sample. A variety of sample types may be utilized in accordance with the present methods including serum, plasma and blood samples. Frequently, the sample is a clinical sample and the mammal is a human. The non-whole PTH fragment may be any of the variety of non-whole PTH fragments as described herein.

[0018] In one aspect, the antibody specifically binds to an epitope comprised in PTH_{1-6}, PTH_{1-8}, PTH_{1-9}, PTH_{1-12}, or PTH_{3-12}, and/or the PTH peptide PTH_{1-15}. Frequently, the antibody specifically binds to a PTH epitope as discussed herein. On occasion, the binding between the antibody and the N-terminal sequence of whole PTH is dependent on the presence of amino acid residues 2-5 or 3-6 of the PTH.

[0019] Although a variety of assay types are contemplated, the present methods frequently assess the complex formed between the whole parathyroid hormone and the antibody via a sandwich or competitive assay format. On occasion, the complex is assessed in a homogeneous or a heterogeneous assay format. Also frequently, the complex is assessed by a format selected from the group consisting of an enzyme-linked immunosorbent assay (ELISA), immunoblotting, immunoprecipitation, radioimmunoassay (RIA), immunostaining, latex agglutination, indirect hemagglutination assay (IHA), complement fixation, indirect immunofluorescent assay (IFA), nephelometry, flow cytometry assay, plasmon resonance assay, chemiluminescence assay, lateral flow immunoassay, u-capture assay, inhibition assay and avidity assay. In a sandwich assay format, the antibody that specifically binds to an N-terminal sequence of whole PTH is used as a first antibody and antibody that is capable of binding to a portion of whole PTH other than the N-terminal sequence which binds to the first antibody is used as a second antibody. Either the first antibody or the second antibody is

frequently attached to a surface and functions as a capture antibody. The attachment can be direct or indirect. In a preferred embodiment, the attachment is provided via a biotin-avidin (or streptavidin) linking pair.

[0020] In another aspect, the physiological level of whole parathyroid hormone is less than 4 pmol/L. Frequently, the physiological level of whole parathyroid hormone is from about 0.2 pmol/L to about 4 pmol/L. Also frequently, the physiological range of whole PTH ranges between about 2 pgm/ml to about 40 pgm/ml. On occasion, the physiological range of whole PTH ranges between about 7 pgm/ml to about 39 pgm/ml.

[0021] In a further embodiment, the present methods may be utilized to measure multiple PTH peptide components, such as a non-whole PTH peptide fragment level and/or a total PTH level, in addition to a whole PTH level. In such embodiments, the methods frequently further comprise comparing at least two parameters selected from the group consisting of the whole PTH level (wPTH), total PTH level, total PTH peptide fragment level, C-terminal PTH fragment level (cPTH), N-terminal PTH fragment level, and mid-terminal PTH fragment level (mPTH). The comparison of parameters is generally in the form of a ratio or proportion. Frequently, the results of said comparison are used to determine whether the mammal, often comprising a human patient, is afflicted with a bone turnover related disorder, or used to monitor bone disease related treatment. Also frequently, the present methods are used to determine or diagnose whether the mammal is afflicted with, or at risk for, adynamic bone disease or severe hyperparathyroidism. Frequently, the present methods are used for clinical management of renal disease subjects and subjects afflicted with osteoporosis, including dialysis patients. Also frequently, the present methods are used for diagnosing primary hyperparathyroidism. Moreover, the present methods are useful for clinical diagnosis and management of subjects having adynamic bone disease induced, in part, through the practice of inappropriate treatment protocols.

[0022] In preferred embodiments of the present comparison the comparison takes many forms. For example, the comparison can be in the form of a ratio or proportion between the whole PTH level versus the total PTH level (*i.e.*, represented by the equation: wPTH / total PTH); between the whole PTH level versus the combined cPTH and mPTH fragment levels (*i.e.*, represented by the equation: wPTH / (cPTH+mPTH)); between the whole PTH level versus the combined cPTH and mPTH fragment levels, wherein double the whole PTH level is subtracted from the combined cPTH and mPTH fragment levels (*i.e.*, represented by the equation: wPTH / ((cPTH-wPTH)+(mPTH-wPTH))); between the whole PTH level versus the total of the combined cPTH and mPTH fragment levels subtracted by the whole PTH level (*i.e.*, represented by the equation: wPTH / (cPTH+mPTH-wPTH)); between the whole PTH level versus the combined whole PTH level, cPTH and mPTH fragment levels (*i.e.*, represented by

the equation: wPTH / (wPTH+cPTH+mPTH)); between the whole PTH level versus the cPTH fragment level (*i.e.*, represented by the equation: wPTH / cPTH); between the whole PTH level versus the mPTH fragment level (*i.e.*, represented by the equation: wPTH / mPTH); between the whole PTH level versus the total PTH level minus the whole PTH level (*i.e.*, represented by the equation: wPTH / (total PTH-wPTH)); or other combinations of the disclosed parameters, including, without limitation, the inverse of each comparison. Moreover, without limitation, in one aspect, the value obtained from determining the total PTH level and subtracting this level from the whole PTH level yields the total PTH fragment level in a sample/subject. The cutoff ranges for each of these comparisons as they are associated with a particular bone turnover, treatment, disease or disorder vary as provided herein (*see e.g.*, Table 2 and accompanying discussion).

[0023] Frequently in the present methods the sample is contacted with one or more isolated antibodies, and wherein each of said one or more isolated antibodies specifically binds one or more PTH peptide fragments selected from the group consisting of: PTH_{39-84}, PTH_{1-34}, PTH_{43-68}, PTH_{7-84}, PTH_{39-68}, PTH_{53-84}, PTH_{65-84}, PTH_{44-68}, PTH_{19-84}, PTH_{23-84}, PTH_{1-38}, PTH_{1-48}, PTH_{1-58}, PTH_{1-68}, and PTH_{1-78}.

[0024] The present methods of measuring multiple PTH components provide a variety of uses. For example, such methods are used for differentiating between a person having substantially normal parathyroid function and having hyperparathyroidism, *e.g.*, primary hyperparathyroidism; monitoring parathyroid related bone disease and treatment; monitoring effects of therapeutic treatment for hyperparathyroidism; diagnosing parathyroid related bone disease; clinical management of renal disease subjects and renal disease related treatments and subjects afflicted with osteoporosis and osteoporosis related treatments.

[0025] The present disclosure further provides kits for carrying out the presently described methods and utilizing the peptides and antibodies as described herein. In one embodiment, a kit for measuring a physiological level of whole parathyroid hormone in a mammalian sample, which kit comprises, in a container, an isolated antibody that specifically binds to an N-terminal sequence of whole parathyroid hormone (PTH) and is capable of detecting said whole PTH at a physiological level in a mammalian sample, with a proviso that said isolated antibody avoids binding to a non-whole PTH fragment.

[0026] In another embodiment, the present disclosure further provides kits for producing an antibody to a parathyroid hormone (PTH) or a PTH peptide, which kits comprise: a) an isolated PTH peptide; b) means for introducing said isolated PTH peptide to a mammal in an amount sufficient to produce an antibody to said PTH peptide; and c) means for recovering said antibody from said mammal. In further embodiment, a kit for producing an antibody to a

parathyroid hormone (PTH) or a PTH peptide is provided which comprises: a) a MAP; b) a means for introducing said MAP to a mammal in an amount sufficient to produce an antibody to a PTH peptide comprised in said MAP; and b) a means for recovering said antibody from said mammal. In a still further embodiment, a kit is provided for producing an antibody to a parathyroid hormone (PTH) or a PTH peptide, which kit comprises: a) a PTH protein or peptide from between PTH_{1-34} and PTH_{1-84}; b) means for introducing said PTH protein or peptide from between PTH_{1-34} and PTH_{1-84} to a mammal in an amount sufficient to produce an antibody to said PTH protein or peptide; c) means for recovering said antibody from said mammal; and c) another specific PTH peptide.

[0027] An isolated PTH peptide in such kits can be any of the variety of PTH peptides as described herein. Frequently, the PTH peptide are conjugated to a carrier to enhance the PTH peptide's immunogenecity, e.g., a carrier protein, which may together form a fusion protein. For example, such PTH peptide is selected from the group consisting of PTH_{1-11}, PTH_{1-13}, PTH_{1-14}, PTH_{1-15}, PTH_{1-16}, PTH_{1-17}, PTH_{1-18}, PTH_{1-19}, PTH_{1-20}, PTH_{1-21}, PTH_{1-22}, PTH_{1-23}, PTH_{1-24}, PTH_{1-25}, PTH_{1-26}, $hPTH_{1-27}$, PTH_{1-28}, PTH_{1-29}, PTH_{1-30}, PTH_{1-31}, PTH_{1-32}, PTH_{1-33}, PTH_{1-34}, PTH_{1-35}, PTH_{1-36}, PTH_{2-5}, PTH_{2-6}, PTH_{2-8}, PTH_{2-9}, PTH_{2-10}, PTH_{2-11}, PTH_{2-12}, PTH_{2-13}, PTH_{2-14}, PTH_{2-15}, PTH_{2-16}, PTH_{2-17}, PTH_{2-18}, PTH_{2-19}, PTH_{2-20}, PTH_{2-21}, PTH_{2-22}, PTH_{2-23}, PTH_{2-24}, PTH_{2-25}, PTH_{2-26}, PTH_{2-27}, PTH_{2-28}, PTH_{2-29}, PTH_{2-30}, PTH_{2-31}, PTH_{2-32}, PTH_{2-33}, PTH_{2-34}, PTH_{2-35}, PTH_{2-36}, PTH_{3-6}, PTH_{3-7}, PTH_{3-9}, PTH_{3-10}, PTH_{3-11}, PTH_{3-12}, PTH_{3-13}, PTH_{3-14}, PTH_{3-15}, PTH_{3-16}, PTH_{3-17}, PTH_{3-18}, PTH_{3-19}, PTH_{3-20}, PTH_{3-21}, PTH_{3-22}, PTH_{3-23}, PTH_{3-24}, PTH_{3-25}, PTH_{3-26}, PTH_{3-27}, PTH_{3-28}, PTH_{3-29}, PTH_{3-30}, PTH_{3-31}, PTH_{3-32}, PTH_{3-33}, PTH_{3-34}, PTH_{3-35}, PTH_{3-36}, PTH_{4-7}, PTH_{4-8}, PTH_{4-9}, PTH_{4-10}, PTH_{4-11}, PTH_{4-13}, PTH_{4-14}, PTH_{4-15}, PTH_{4-16}, PTH_{4-17}, PTH_{4-18}, PTH_{4-19}, PTH_{4-20}, PTH_{4-21}, PTH_{4-22}, PTH_{4-23}, PTH_{4-24}, PTH_{4-25}, PTH_{4-26}, PTH_{4-27}, PTH_{4-28}, PTH_{4-29}, PTH_{4-30}, PTH_{4-31}, PTH_{4-32}, PTH_{4-33}, PTH_{4-34}, PTH_{4-35}, PTH_{4-36}, PTH_{5-8}, PTH_{5-9}, PTH_{5-11}, PTH_{5-12}, PTH_{5-13}, PTH_{5-14}, PTH_{5-15}, PTH_{5-16}, PTH_{5-17}, PTH_{5-18}, PTH_{5-19}, PTH_{5-20}, PTH_{5-21}, PTH_{5-22}, PTH_{5-23}, PTH_{5-24}, PTH_{5-25}, PTH_{5-26}, PTH_{5-27}, PTH_{5-28}, PTH_{5-29}, PTH_{5-30}, PTH_{5-31}, PTH_{5-32}, PTH_{5-33}, PTH_{5-34}, PTH_{5-35}, PTH_{5-36}, and PTH_{5-37}.

[0028] The presently contemplated kits may also provide an immunogen comprising a PTH peptide as described herein, together with an immune response potentiator. On occasion, the immune response potentiator is selected from the group consisting of Bacille Calmette-Guerin (BCG), Corynebacterium Parvum, Brucella abortus extract, glucan, levamisole, tilorone, an enzyme and a non-virulent virus.

[0029] The present disclosure further provides methods for producing an antibody to a parathyroid hormone (PTH) or a PTH peptide. In one embodiment, such method comprises: a) introducing an isolated PTH peptide to a mammal in an amount sufficient to produce an antibody to said PTH peptide; and b) recovering said antibody from said mammal. Another

frequent method for producing an antibody to a parathyroid hormone (PTH) or a PTH peptide comprises: a) introducing a MAP to a mammal in an amount sufficient to produce an antibody to a PTH peptide comprised in said MAP; and b) recovering said antibody from said mammal. In one aspect, the present disclosure provides antibodies to a PTH or a PTH peptide produced by these methods. In a related embodiment, a method is provided for producing an antibody to a parathyroid hormone (PTH) or a PTH peptide, which method comprises: a) introducing a PTH protein or peptide from between PTH_{1-34} and PTH_{1-84} to a mammal in an amount sufficient to produce an antibody to said PTH protein or peptide; b) recovering said antibody from said mammal; and c) affinity purifying a PTH antibody that specifically binds to an epitope comprised in a PTH peptide using said PTH peptide. In a further embodiment, the present disclosure provides an antibody to a PTH or a PTH peptide produced by such methods.

V. BRIEF DESCRIPTION OF THE DRAWINGS

[0030] FIGURE 1 is a diagrammatic view of whole human PTH (SEQ ID NO: 1).

[0031] FIGURE 2 is a diagrammatic view of a wPTH assay using the present antibody as a tracer element.

[0032] FIGURE 3 is a diagrammatic view of a wPTH assay using the present antibody as a capture element.

[0033] FIGURE 4 is a graph showing a standard curve for a wPTH assay.

[0034] FIGURE 5 is a graph comparing a conventional I-PTH assay with the present wPTH assay for healthy normal persons with "normal" PTH values.

[0035] FIGURE 6 is a diagrammatic view showing interference from non (1-84) PTH fragments in conventional I-PTH assays.

[0036] FIGURE 7 is a graph comparing a conventional I-PTH assay with the present wPTH assay for patients with chronic uremia.

[0037] FIGURE 8 is a graph showing the distribution of values for healthy normal persons, patients with primary hyperparathyroidism, and patients with chronic uremia.

[0038] FIGURE 9 is a diagrammatic view showing how PIN blocks the action of wPTH at the receptor level, thereby making the person insensitive to the biological effects of wPTH.

[0039] FIGURE 10 is a graph demonstrating complete cross-reactivity of wPTH and PIN in a total PTH assay used in the present invention.

[0040] FIGURE 11 is a graph demonstrating how the whole PTH assay used in the present invention does not detect to PIN.

[0041] FIGURE 12 is a graph demonstrating how PIN is an *in vivo* inhibitor of wPTH.

[0042] FIGURE 13 illustrates comparison of the recognition of hPTH 1-84 and hPTH 7-84 by the Nichols I-PTH assay. The Nichols I-PTH assay does not differentiate between hPTH 1-84 (solid line) and hPTH 7-84 (dashed line).

[0043] FIGURE 14 illustrates comparison of the recognition of hPTH 1-84 and hPTH 7-84 by the Whole PTH assay. Unlike the Nichols I-PTH assay, the Whole PTH assay does discriminate between hPTH 1-84 (solid line) and hPTH 7-84 (dashed line). Concentrations of hPTH 7-84 as high as 10,000 pg were undetectable.

[0044] FIGURE 15 illustrates comparison of the effect of hPTH 1-84 or hPTH 7-84 on cAMP production in ROS 17.2 cells. Unlike hPTH 7-84, hPTH 1-84 increased cAMP production in a dose-dependent manner. cAMP increased from 18.1 $\pm$1.2 to 738 $\pm$4.1 nmol/well after treatment with 10^{-8}mol/L hPTH 1-84. The same concentration of hPTH 7-84 had no effect.

[0045] FIGURE 16 illustrates comparison of the effects of hPTH 1-84 or hPTH 1-84 plus hPTH 7-84 on (A) glomerular filtration rate (GFR) and (B) fractional excretion of phosphorus (FE_{po4}). Control and treatment periods are denoted by open and closed bars, respectively. The phosphaturia induced by hPTH 1-84 was decreased by 50.2% ($P<0.05$) when animals were treated simultaneously with 7-84 PTH, despite a significant increase in GFR ($P<0.005$).

[0046] FIGURE 17 illustrates comparison of PTH values in plasma from uremic patients using the Nichols "intact" PTH assay (■) versus the Whole PTH assay (●). Plasma PTH values are uniformly higher when measured with the Nichols "intact" PTH assay than with the Whole PTH assay. The median PTH values were 523 vs. 344 pg/mL, respectively ($P<0.001$).

[0047] FIGURE 18 illustrates effects of plasma calcium on PTH degradation in dialysis patients. The percentage of non-(1-84) PTH fragment (likely hPTH 7-84) correlates positively with plasma calcium ($P<0.02$) ($r=0.638$; $P=0.0025$; $N=20$).

[0048] FIGURE 19 illustrates comparison of plasma PTH levels in renal transplant patients using Nichols I-PTH and Whole PTH assays. PTH values are higher when measured with the Nichols I-PTH assay ($P>0.005$).

[0049] FIGURE 20 illustrates intracellular PTH content on parathyroid glands from uremic patients. The 41.8 ± 3.2% of the total PTH, measured by the I-Nichols assay (expressed as 100%), represents the non-(1-84) PTH fragment "likely" hPTH 7-84 (?). the 1-84 PTH molecule was measured with the Whole PTH assay (¦).

VI. DETAILED DESCRIPTION OF THE INVENTION

A. DEFINITIONS

[0050] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as is commonly understood by one of ordinary skill in the art to which this invention belongs. All patents, patent applications (published or unpublished), and other publications referred to herein are incorporated by reference in their entirety. If a definition set forth in this section is contrary to or otherwise inconsistent with a definition set forth in the patents, applications, published applications and other publications that are herein incorporated by reference, the definition set forth in this section prevails over the definition that is incorporated herein by reference.

[0051] As used herein, "a" or "an" means "at least one" or "one or more."

[0052] As used herein, "antibody" is used in the broadest sense. Therefore, an "antibody" can be naturally occurring or man-made such as monoclonal antibodies produced by conventional hybridoma technology and/or a functional fragment thereof. Antibodies of the present invention comprise monoclonal and polyclonal antibodies as well as fragments containing the antigen-binding domain and/or one or more complementarity determining regions of these antibodies.

[0053] As used herein, "monoclonal antibody" refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the antibodies comprising the population are identical except for possible naturally occurring mutations that are present in minor amounts. As used herein, a "monoclonal antibody" further refers to functional fragments of monoclonal antibodies.

[0054] As used herein, "mammal" refers to any of the mammalian class of species. Frequently, the term "mammal," as used herein, refers to humans, human subjects or human patients.

[0055] As used herein, "whole parathyroid hormone (PTH)" or "wPTH" refers to the complete molecule of PTH. This term is not species-specific unless otherwise designated. For purposes herein, the name "parathyroid hormone (PTH)" is used herein, although all other names are contemplated. It is intended to encompass whole PTH with conservative amino acid substitutions that do not substantially alter its biological activity. Suitable conservative

substitutions of amino acids are known to those of skill in this art and may be made generally without altering the biological activity of the resulting molecule. Those of skill in this art recognize that, in general, single amino acid substitutions in non-essential regions of a polypeptide do not substantially alter biological activity (*see, e.g.*, Watson et al., MOLECULAR BIOLOGY OF THE GENE, 4th Edition, 1987, The Bejamin/Cummings Pub. Co., p.224).

[0056] As used herein, "parathyroid hormone (PTH) agonist," "cyclase activating PTH" or "CAP" refers to the complete molecule of PTH or a fragment, derivative or analog thereof that stimulates osteoclasts formation and bone turnover to increase blood calcium levels. PTH agonist further refers to peptides which have PTH agonist properties. Other names of PTH include parathormone and parathyrin. For purposes herein, the name "parathyroid hormone (PTH)" is used herein, although all other names are contemplated. It is intended to encompass PTH agonist with conservative amino acid substitutions that do not substantially alter its biological activity. Suitable conservative substitutions of amino acids are known to those of skill in this art and may be made generally without altering the biological activity of the resulting molecule. Those of skill in this art recognize that, in general, single amino acid substitutions in non-essential regions of a polypeptide do not substantially alter biological activity (see, e.g., Watson et al., MOLECULAR BIOLOGY OF THE GENE, 4th Edition, 1987, The Bejamin/Cummings Pub. co., p.224). PTH agonist assay values may be obtained by measuring a sample with a Scantibodies Whole PTH Assay or a Scantibodies CAP Assay or a 3rd generation PTH Assay or a Nichols BioIntact PTH assay or an Immutopics Human Bioactive PTH assay.

[0057] As used herein, the term "total PTH" refers to a total accounting of whole PTH levels in addition to PTH fragment levels. Moreover, this term is not species-specific unless otherwise designated.

[0058] As used herein, the term "PIN" refers to PTH fragments that have PTH antagonistic or inhibiting properties. Therefore, although occasionally of concurrent scope, a reference to PTH fragments, as provided herein, is not intended to be limited to PIN.

[0059] As used herein, a "PTH fragment" is a PTH peptide that comprises a non-whole contiguous portion of an entire PTH protein. A reference to a PTH fragment as herein includes C-terminal, mid-terminal fragments and PIN, unless otherwise indicated. Moreover, this term is not species-specific unless otherwise designated.

[0060] As used herein, "treatment" means any manner in which the symptoms of a condition, disorder or disease are ameliorated or otherwise beneficially altered. Treatment also encompasses any pharmaceutical use of the compositions herein.

[0061] As used herein, "disease or disorder" refers to a pathological condition in an organism resulting from, e.g., infection or genetic defect, and characterized by identifiable symptoms.

[0062] As used herein, "high bone turnover" refers to the bone turnover rate as being above a normal bone turnover rate in a subject and is one of the symptoms manifested in subjects having hyperparathyroidism. While not bound by theory, a subject afflicted with severe hyperparathyroidism has a higher bone turnover rate than the same subject afflicted with mild hyperparathoidism, however, both having a high bone turnover rate as compared with a normal subject and a subject afflicted with adynamic bone disease.

[0063] As used herein, the term "subject" is not limited to a specific species or sample type. For example, the term "subject" may refer to a patient, and frequently a human patient. However, this term is not limited to humans and thus encompasses a variety of mammalian species.

[0064] As used herein, "afflicted" as it relates to a disease or disorder refers to a subject having or directly affected by the designated disease or disorder.

[0065] As used herein the term "sample" refers to anything which may contain an analyte for which an analyte assay is desired. The sample may be a biological sample, such as a biological fluid or a biological tissue. Examples of biological fluids include urine, blood, plasma, serum, saliva, semen, stool, sputum, cerebral spinal fluid, tears, mucus, amniotic fluid or the like. Biological tissues are aggregate of cells, usually of a particular kind together with their intercellular substance that form one of the structural materials of a human, animal, plant, bacterial, fungal or viral structure, including connective, epithelium, muscle and nerve tissues. Examples of biological tissues also include organs, tumors, lymph nodes, arteries and individual cell(s).

[0066] As used herein the term "avoids binding" refers to the specificity of particular antibodies or antibody fragments. Antibodies or antibody fragments that avoid binding a particular moiety generally contain a specificity such that a large percentage of the particular moiety would not be bound by such antibodies or antibody fragments. This percentage generally lies within the acceptable cross reactivity percentage with interfering moieties of assays utilizing antibodies directed to detecting a specific target. Frequently, antibodies or antibody fragments of the present disclosure avoid binding greater than about 90% of an interfering moiety, although higher percentages are clearly contemplated and preferred. For example, antibodies or antibody fragments of the present disclosure avoid binding about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, and about 99% or more of an interfering moiety. Less occasionally, antibodies or antibody

fragments of the present disclosure avoid binding greater than about 70%, or greater than about 75%, or greater than about 80%, or greater than about 85% of an interfering moiety. Although not bound by theory, as contemplated herein, an interfering moiety may comprise a non-whole PTH fragment.

[0067] As used herein the term "physiological level of whole PTH" refers generally to the average concentration of whole PTH present in a mammal, e.g., a human, expressed in pmol/L, or another suitable measurement unit (*e.g.*, pgm/ml). *See, e.g.,* Woodhead, J.S., Clin. Biochem. 23, 17 (1990). In one aspect, the physiological range of whole PTH ranges between about 0.2 pmol/L to about 4 pmol/L, or about 2 pgm/ml to about 40 pgm/ml. On occasion, the physiological range of whole PTH can range between about 7 pgm/ml to about 39 pgm/ml. Although specific ranges are described herein as representative of a physiological range, one of skill in the art would understand that the physiological level of whole PTH may lie outside of the presently disclosed ranges in certain subjects. Nevertheless, the compositions and methods provided herein are useful to detect discreet concentrations of whole PTH and have sensitivities within the physiological range as provided herein.

[0068] As used herein, the term "N-terminal" refers to the amino terminus of a PTH polypeptide having a free amino group. With reference to a PTH fragment, an N-terminal PTH fragment refers to a non-whole contiguous portion of PTH having an intact N-terminal. An "intact N-terminal" as used herein refers to PTH or a PTH fragment having an intact 1st position of $PTH_{1\text{-}84}$. This first position is also referred to herein as an "original N-terminus" or an "original N-terminal."

[0069] As used herein, the term "C-terminal" refers to the carboxyl terminus of a PTH polypeptide having a free carboxyl group. With reference to a PTH fragment, a C-terminal PTH fragment refers to a non-whole contiguous portion of PTH having an intact C-terminal. An "intact C-terminal" as used herein refers to PTH or a PTH fragment having an intact 84th position of $PTH_{1\text{-}84}$. This 84th position is also referred to herein as an "original C-terminus" or an "original C-terminal."

[0070] As used herein, the term "mid-terminal PTH fragment" refers to a non-whole contiguous portion of PTH having neither an intact N-terminal nor an intact C-terminal. These types of PTH fragments may also be referred to herein as "mid-terminus fragments."

[0071] As used herein, the term "specifically binds" refers to the specificity of an antibody such that it preferentially binds to a defined target. Recognition by an antibody of a particular target in the presence of other potential targets is one characteristic of such binding. Specific binding of the presently contemplated antibodies to particular PTH targets is measured through known methods utilizing the tools provided herein.

[0072] As used herein, "stringency" of hybridization reactions is readily determinable by one of ordinary skill in the art, and generally is an empirical calculation dependent upon probe length, washing temperature, and salt concentration. In general, longer probes require higher temperatures for proper annealing, while shorter probes need lower temperatures. Hybridization generally depends on the ability of denatured nucleic acid sequences to reanneal when complementary strands are present in an environment below their melting temperature. The higher the degree of desired homology between the probe and hybridizable sequence, the higher the relative temperature that can be used. As a result, it follows that higher relative temperatures would tend to make the reaction conditions more stringent, while lower temperatures less so. For additional details and explanation of stringency of hybridization reactions, see Current Protocols in Molecular Biology (Ausubel et al. eds., Wiley Interscience Publishers, 1995); Molecular Cloning: A Laboratory Manual (J. Sambrook, E. Fritsch, T. Maniatis eds., Cold Spring Harbor Laboratory Press, 2d ed. 1989); Wood et al., *Proc. Natl. Acad. Sci. USA*, 82:1585-1588 (1985).

[0073] As used herein the term "isolated" refers to material removed from its original environment, and is altered from its natural state. For example, an isolated polypeptide could be coupled to a carrier, and still be "isolated" because that polypeptide is not in its original environment.

[0074] The present disclosure encompasses antigens, antibodies and methods of producing antibodies that have a particular specificity to target proteins and/or peptides which contain a specific amino acid residue or multiple amino acid residues, in a series or otherwise. The specific amino acid residue(s) may be located in the N-terminal region of a proteins or peptide or in the C-terminal region. Moreover the specific amino acid residue(s) may be located in a region between the N-terminal and C-terminal regions of a protein or peptide. Occasionally, when there is more than one specific amino acid residue, such residues may be dispersed in any one or more of the N-terminal, C-terminal, between these two regions, and/or in all of these regions.

[0075] In disclosing the present invention, one should remember that there are a number of closely analogous, species dependent forms of PTH. The amino acid sequence of hPTH is shown in FIGURE 1. However, for rat PTH, mouse PTH, bovine PTH, canine PTH, horse PTH or porcine PTH, for example, one finds the substitutions at some of the amino acids in the hPTH sequence. For the purposes of the present invention, one can use interchangeably antibodies or antibody fragments to forms of these PTHs, although it is preferred to use an antibody with specificity for PTH having a sequence matching the species in which the PTH measurements are made.

B. PARATHYROID HORMONE FRAGMENTS

[0076] In general, a PTH fragment of the present invention comprises a non-whole contiguous portion of PTH having an amino acid sequence as set forth in SEQ ID NOs: 1 2, 3, 4, 5, 6, and/or 7 (PTH_{1-84}), or a nucleic acid encoding said portion of PTH. A PTH fragment may have the following characteristics: a) the N-terminal amino acid residue of said PTH fragment starts at any position spanning position 1 through position 80 of said PTH_{1-84}; b) the C-terminal amino acid residue of said PTH fragment ends at any position spanning position 4 through position 84 of said PTH_{1-84}; and c) said PTH fragment has a minimal length of three amino acid residues. Preferably, the PTH fragment is in the form of a pharmaceutical composition.

[0077] PTH fragments of the present invention are organized into three categories: N-terminal, C-terminal, and mid-terminal PTH fragments. As further described herein, N-terminal fragments comprise a non-whole contiguous portion of PTH having an intact N-terminus, but not an intact original C-terminus. As also described herein, C-terminal fragments comprise a non-whole contiguous portion of PTH having an intact C-terminus, but not an intact original N-terminus. Moreover, as further described herein, mid-terminal fragments comprise a non-whole contiguous portion of PTH having neither an intact original C-terminus, nor intact original N-terminus. All mammalian sources/sequences of PTH are contemplated.

[0078] In one embodiment, PTH fragments comprise a subset of cyclase inactive PTH. However, in light of the present description, a variety of other PTH fragments are contemplated, ascertainable and useful in the present compositions, kits and methods. Importantly, PTH_{7-84} represents a member of the group of PTH fragments currently contemplated. The present disclosure further contemplates large inactive PTH fragments in the description of PTH fragments.

[0079] In one embodiment, the N-terminal amino acid residue of the PTH fragment starts at any defined position spanning position 2 through position 70 of said PTH_{1-84}. The C-terminal amino acid residue of said PTH fragment ends at any defined position spanning position 35 through position 84 of said PTH_{1-84}. Therefore, for example, fragments ranging from PTH_{2-84} to PTH_{34-84} to PTH_{70-84} are included as C-terminal fragments. Mid-terminal PTH fragments are also contemplated, for example, ranging within PTH_{39-68} or PTH_{44-68}. For example, mid-terminal PTH fragments having their N-terminal beginning around position 44 of said PTH_{1-84} and their C-terminal ending around position 68 of said PTH_{1-84} are included in the present description. Without being bound by theory, a mid-terminal PTH fragment does not include position 1, nor position 84 of said PTH_{1-84}, but rather falls within these positions.

[0080] In a specific embodiment, the PTH fragment is a protein or a peptide, or a nucleic acid encoding said protein or peptide, selected from the group consisting of PTH_{2-84}, PTH_{3-84}, PTH_{4-84}, PTH_{5-84}, PTH_{6-84}, PTH_{7-84}, PTH_{8-84}, PTH_{9-84}, PTH_{10-84}, PTH_{11-84}, PTH_{12-84}, PTH_{13-84}, PTH_{14-84}, PTH_{15-84}, PTH_{16-84}, PTH_{17-84}, PTH_{18-84}, PTH_{19-84}, PTH_{20-84}, PTH_{21-84}, PTH_{22-84}, PTH_{23-84}, PTH_{24-84}, PTH_{25-84}, PTH_{26-84}, PTH_{27-84}, PTH_{28-84}, PTH_{29-84}, PTH_{30-84}, PTH_{31-84}, PTH_{32-84}, and PTH_{33-84}. In another specific embodiment, the PTH fragment is a protein or a peptide, or a nucleic acid encoding said protein or peptide, selected from the group consisting of PTH_{7-69}, PTH_{7-70}, PTH_{7-71}, PTH_{7-72}, PTH_{7-73}, PTH_{7-74}, PTH_{7-75}, PTH_{7-76}, PTH_{7-77}, PTH_{7-78}, PTH_{7-79}, PTH_{7-80}, PTH_{7-81}, PTH_{7-82}, PTH_{7-83} and PTH_{7-84}.

[0081] In another embodiment, the PTH fragment is a protein or a peptide, or a nucleic acid encoding said protein or peptide, selected from the group consisting of PTH_{34-84}, PTH_{35-84}, PTH_{36-84}, PTH_{37-84}, PTH_{38-84}, PTH_{39-84}, PTH_{40-84}, PTH_{41-84}, PTH_{42-84}, PTH_{43-84}, PTH_{44-84}, PTH_{45-84}, PTH_{46-84}, PTH_{47-84}, PTH_{48-84}, PTH_{49-84}, PTH_{50-84}, PTH_{51-84}, PTH_{52-84}, PTH_{53-84}, PTH_{54-84}, PTH_{55-84}, PTH_{56-84}, PTH_{57-84}, PTH_{58-84}, PTH_{59-84}, PTH_{60-84}, PTH_{61-84}, PTH_{62-84}, PTH_{63-84}, PTH_{64-84}, PTH_{65-84}, PTH_{66-84}, PTH_{67-84}, PTH_{68-84}, PTH_{69-84}, and PTH_{70-84}.

[0082] In a further embodiment, the PTH fragment is a protein or a peptide, or a nucleic acid encoding said protein or peptide, selected from the group consisting of PTH_{7-60}, PTH_{8-60}, PTH_{9-60}, PTH_{10-60}, PTH_{11-60}, PTH_{12-60}, PTH_{13-60}, PTH_{14-60}, PTH_{15-60}, PTH_{16-60}, PTH_{17-60}, PTH_{18-60}, PTH_{19-60}, PTH_{20-60}, PTH_{21-60}, PTH_{22-60}, PTH_{23-60}, PTH_{24-60}, PTH_{25-60}, PTH_{26-60}, PTH_{27-60}, PTH_{28-60}, PTH_{29-60}, PTH_{30-60}, PTH_{31-60}, PTH_{32-60}, PTH_{33-60}, PTH_{34-60}, PTH_{35-60}, PTH_{36-60}, PTH_{37-60}, and PTH_{38-60}, PTH_{39-60}, PTH_{40-60}, PTH_{41-60}, PTH_{42-60}, PTH_{43-60}, PTH_{44-59}, PTH_{44-60}, PTH_{45-60}, PTH_{46-60}, PTH_{47-60}, and PTH_{48-60}, and other mid-terminal PTH fragments as described herein. In another specific embodiment, the PTH fragment is a protein or a peptide, or a nucleic acid encoding said protein or peptide, selected from the group consisting of PTH_{13-34}, PTH_{7-53}, PTH_{8-53}, PTH_{9-53}, PTH_{10-53}, PTH_{11-53}, PTH_{12-53}, PTH_{13-53}, PTH_{14-53}, PTH_{15-53}, PTH_{16-53}, PTH_{17-53}, PTH_{18-53}, PTH_{19-53}, PTH_{20-53}, PTH_{21-53}, PTH_{22-53}, PTH_{23-53}, PTH_{24-53}, PTH_{25-53}, PTH_{26-53}, PTH_{27-53}, PTH_{28-53}, PTH_{29-53}, PTH_{30-53}, PTH_{31-53}, PTH_{32-53}, PTH_{33-53}, PTH_{34-53}, PTH_{35-53}, PTH_{6-53}, PTH_{37-53}, and PTH_{38-53}, and other mid-terminal PTH fragments as described herein.

[0083] In another preferred embodiment, a PTH fragment comprises, or an antibody specifically binds, a PTH peptide fragment selected from the group consisting of: PTH_{39-84}, PTH_{1-34}, PTH_{43-68}, PTH_{7-84}, PTH_{39-68}, PTH_{53-84}, PTH_{65-84}, PTH_{44-68}, PTH_{19-84}, PTH_{23-84}, PTH_{1-68}, or a combination of two or more from this group. This group may further comprise a PTH peptide fragment having an N-terminus starting between position 39 to 65 of PTH_{1-84}, and having a C-terminal position ending at position 84 of PTH_{1-84}. In a particularly preferred

embodiment, a PTH peptide fragment comprises a PTH fragment present and detectable in nature.

[0084] In another embodiment, the PTH fragment is a protein or a peptide, or a nucleic acid encoding said protein or peptide having an intact N-terminus, for example, without limitation, PTH_{1-38}, PTH_{1-48}, PTH_{1-58}, PTH_{1-68}, PTH_{1-78}, amongst other intact N-terminal PTH fragments.

[0085] The PTH fragment can have any suitable length and may have PTH agonizing or antagonizing activity, although PTH agonizing or antagonizing activity is not required of the present PTH fragments. For example, the PTH fragment can have a length of 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82 or 83 amino acid residues.

C. PTH RATIOS - WHOLE PTH AND PTH FRAGMENTS

[0086] An important discovery leading to the present invention is that adynamic bone loses its capacity to buffer calcium and phosphate as the bones are shut down. In subjects afflicted with such conditions, they are unable to effectively buffer calcium as it enters their bodies through their diet. This calcium enters the blood stream and is thereafter shuttled to the soft tissues. The parathyroid gland is particularly subject to, and detrimentally affected by, this influx of calcium and thereby produces PTH fragments rather than, or in addition to, the active form of PTH. *See, e.g.*, Mayer GP, et al., *Endocrinology* 104: 1778–1784 (1979); D'Amour P, et al., *J. Clin. Endocrinol. Metab.* 74: 525–532 (1992); D'Amour P, et al., *J. Bone Miner. Res.* 11: 1075–1085 (1996); Cardinal, H., et al., *J. Clin. Endocrinol. Metab.* 83: 3839-44 (1998). Accordingly, in subjects with adynamic bone, the concentration and production of PTH fragments is increased. In light of this and other related information, the measurement of PTH fragment levels, and particularly in conjunction with the measurement of whole PTH, can be used effectively to differentiate subjects having adynamic bone versus those having normal bone and high bone turnover rates.

[0087] The present disclosure includes these findings in the presentation of peptides, antibodies, methods and kits for the measurement of PTH levels. In one preferred embodiment, the present methods utilize a ratio of whole PTH to total PTH, wherein the total PTH level comprises whole PTH plus PTH fragments in addition to PTH_{7-84}, such as other PTH fragments described herein (*i.e.*, whole PTH / total PTH ratio). This ratio contains an increased total PTH concentration as compared with a PTH ratio that comprises a total PTH level reflecting measurement of whole PTH plus PTH fragments that specifically bind to an antibody generated

from PTH_{7-84} peptide. Accordingly, the CAP/PTH fragment ratio according is generally lower than the CAP/(CAP + PTH_{7-84}) ratio. Until presently, recognition of the predictive and therapeutic benefits of PTH ratios have been unrecognized. *See, e.g.*, Martine-Esther Cohen Solal, *et al., J. Clin. Endocrinol. Metab.* 73: 516-524 (1991) (concluding that the measurement of whole PTH is "superior to C-terminal and midregion assays for the prediction of histological type bone diseases.").

[0088] In one embodiment, a total PTH assay is utilized wherein an antibody specific for PTH_{44-68} is utilized in addition to an antibody specific for PTH_{52-84} in addition to other potential antibodies to determine a total PTH level.

[0089] The present disclosure also provides a more therapeutically predictive PTH ratios involving whole PTH levels, PTH fragment levels, and occasionally total PTH levels. The total PTH levels in this ratio include PTH fragments in addition to large PTH N-terminal fragments such as PTH_{7-84}. These PTH fragments include a category of PTH fragments referred to elsewhere in the present disclosure as large inactive fragments and are not necessarily C-terminal nor N-terminal PTH fragments as described herein. As provided herein, PTH fragments included in the present total PTH determinations include PTH_{7-84}, in addition to other PTH fragments. Thus, an important aspect of the present enhanced PTH ratio involves monitoring a majority, and more preferably all, of the circulating PTH fragments in a subject such that a total PTH assay will measure N-terminal, C-terminal and mid-terminal PTH fragments. In a related aspect, the fragments measured in the enhanced ratio include PTH_{7-84} in addition to other PTH fragments as described herein.

[0090] For example, an enhanced PTH ratio comprised of whole PTH versus total PTH (measuring most or all circulating PTH fragments in addition to whole PTH) will generally provide a lower percentage as compared with a PTH ratio measuring a total PTH ratio comprising whole PTH versus "total" PTH (consisting of whole PTH plus large N-terminal PTH fragments such as PTH_{7-84}). This lower percentage is due to the measurement of additional fragments in addition to PTH_{7-84} as comprising the total PTH. Prior PTH totals generally provided lower numbers than PTH totals including PTH fragments in addition to PTH_{7-84}. These totals were lower because PTH fragments other than large N-terminal fragments were not recognized. The present invention acknowledges this previous flaw in PTH totals in addition to the enhanced predictive and therapeutic benefits of ratios derived from measuring PTH fragments in addition to circulating N-terminal fragments.

[0091] The presently contemplated PTH ratios are useful to provide cutoff valuations to determine whether a subject suffers from adynamic bone disease (ADN), mild hyperparathyroidism (mild HPT) or severe hyperparathyroidism (severe HPT). Frequently, the

present ratios are useful for initial diagnosis. However, these ratios may be equally useful to monitor and guide therapy for subjects. In one preferred embodiment, the present ratios are utilized in conjunction with measurement of CAP and/or whole PTH levels. Table 1 below provides a reference table for bone turnover rates as they relate to whole PTH levels and PTH ratios consisting of PTH_{1-84} versus PTH_{7-84}.

TABLE 1

	Normal Turnover	High Turnover	Adynamic Bone Disease
Ratio PTH_{1-84}/PTH_{7-84}	Between about 1.4 to about 2.0	Greater than about 2.0	Less than about 1.4
PTH level PTH_{1-84} (pg/ml)	Between about 90-170	Greater than about 170	Less than about 90

[0092] While not bound by theory, in light of current standards and practices, reliance solely on the values and ratios in Table 1 for determination and differentiation between the three listed bone turnover rate categories would be misplaced without reference to additional data including, for example, bone histology information and/or additional fragment related information (as provided herein).

[0093] Example ratios include variations of PTH parameters comprised of whole PTH (wPTH), C-terminal PTH (cPTH), and mid-terminus PTH fragments (mPTH). Depending on the antibodies utilized, cross-reactivity between these parameters may be witnessed (and corrected). A non-limiting list of example ratios of the present disclosure include wPTH/cPTH, wPTH/mPTH, wPTH/(cPTH+mPTH), wPTH/(cPTH-wPTH)+(mPTH-wPTH), wPTH/(cPTH+mPTH-wPTH), wPTH/(wPTH+mPTH+cPTH), cPTH/mPTH, mPTH/cPTH, cPTH/wPTH, mPTH/wPTH, and so forth. One of skill in the art would recognize that the inverse of these PTH ratios and other combinations of these parameters are equally suitable in the present methods. Moreover, CAP may be utilized as a PTH ratio parameter and used in conjunction with whole PTH levels, total PTH levels and/or PTH fragment levels in the contemplated ratios.

[0094] For example, Table 2 below provides a series of PTH ratio cutoff values. Antibodies specific for whole PTH (wPTH), mid-terminus PTH fragments (mPTH), and C-terminal PTH (cPTH) fragments and corresponding histology data are utilized to generate PTH level raw data providing the base values for ratio cutoffs. Each of the ratios presented below are generated based on the levels of cPTH, mPTH and/or wPTH as depicted.

TABLE 2

Ratio composition	Cutoff (about)	Indication
wPTH/(cPTH+mPTH)	<0.020	ADN
wPTH/(cPTH+mPTH)	>0.020	Severe HPT
wPTH/(cPTH+mPTH)	0.020	Mild HPT
wPTH/((cPTH-wPTH)+(mPTH-wPTH))	<0.0185	ADN
wPTH/((cPTH-wPTH)+(mPTH-wPTH))	>0.0185	Severe HPT
wPTH/((cPTH-wPTH)+(mPTH-wPTH))	>0.0185	Mild HPT
wPTH/(cPTH+mPTH-wPTH)	<0.020	ADN
wPTH/(cPTH+mPTH-wPTH)	>0.020	Severe HPT
wPTH/(cPTH+mPTH-wPTH)	0.020	Mild HPT
wPTH/(wPTH+mPTH+cPTH)	<0.0175	ADN
wPTH/(wPTH+mPTH+cPTH)	>0.0175	Severe HPT
wPTH/(wPTH+mPTH+cPTH)	>0.0175	Mild HPT
wPTH/cPTH	<0.103	ADN
wPTH/cPTH	>0.103	Severe HPT
wPTH/cPTH	0.103	Mild HPT
wPTH/mPTH	<0.0225	ADN
wPTH/mPTH	>0.0225	Severe HPT
wPTH/mPTH	>0.0225	Mild HPT

[0095] While Table 2 presents cutoff ranges for each of three clinical indications, variation for each may exist. The term "about" is used with each cutoff as a particularly preferred value range having slight inherent variation which may include the cutoff point as well. One of skill in the art would understand that PTH assay parameters could be changed such that the cutoff point may vary from that provided above; such variation falls within the scope of the present disclosure. In one embodiment, the cutoff point represents a median cutoff

value for an indication. In a preferred embodiment, the cutoff point represents a range below or above which a majority or all subjects having a particular indication fall within.

[0096] There is a tremendous need to be able to non invasively separate the dialysis patients with ADN from those suffering from high bone turnover to avoid over treatment of ADN dialysis patients. Over treatment of dialysis patients with ADN is a frequent occurrence under presently utilized methods. For example, calcium based phosphate binders such as Zemplar® and Calcijex® (Abbott Laboratories), for example, have been used to treat dialysis patients. Under the treatment protocols utilized and recommended, these patients are at a great risk of over treatment due to inaccurate measurement of PTH levels (including wPTH and PTH fragment levels). For example, the proportion of dialysis patients treated with calcium based phosphate binders that become afflicted with ADN rose sharply during the time spanning 1995 to 2000 from 12% to 48% of such patients. *See, e.g.*, Malluche, H.H., *The Importance of Bone Health in ESRD: Out of the Frying Pan, Into the Fire?*, World Congress on Nephrology, Berlin, Germany (June 2003) (based on unpublished data). It is postulated that this increase is due, in large part, to over treatment of dialysis patients; and this over treatment of dialysis patients is due, in turn, to ineffective PTH level monitoring (including whole PTH and PTH fragment levels). Moreover, K/DOQI recommends whole PTH as the only marker useful for separating ADN from HBT dialysis patients. *See* K/DOQI Clinical Practice Guidelines for Bone Metabolism and Disease in Chronic Kidney Disease, *Draft Guideline Statements and Treatment Algorithms* (February 2003). However, whole PTH levels fail to consistently separate ADN from HBT dialysis patients. *See* Qi, Q, *et al.*, *Am. J. Kidney Dis.* 26:622-31 (1995); Quarles, LD, *et al.*, *J. Clin. Endocrinol. Metab.* 75:145-150 (1992). It is recognized herein that such guidelines would appear to propagate the over treatment phenomenon. Accordingly, the present compositions and methods illustrate the effectiveness of separating ADN from HBT dialysis patients via PTH ratio results, rather than via measurement of whole PTH levels alone, and their use for routine clinical management of renal disease, osteoporosis, and/or dialysis patients.

D. PTH ASSAY LOCATIONS

[0097] The presently contemplated methods may be performed in a variety of settings and by a variety of entities. However, in general, the present methods and materials may be made available in a health care setting. Frequently, the present methods, *e.g.*, determining and monitoring of PTH levels and ratios as described herein, are utilized in the clinical management of disease or disorders in a subject by a care provider or clinical laboratory. A health care setting, as used herein, includes clinical laboratories, doctor's offices, hospitals, health management organization facilities, and outpatient care facilities, amongst a variety of other nontraditional settings useful for the delivery of care and subject testing.

E. PTH SEQUENCES

[0098] The present disclosure contemplates the use of parathyroid hormone peptides, peptide fragments, polynucleotides encoding whole PTH or PTH fragment peptides, and antibodies that specifically bind whole PTH and/or PTH fragments derived from a variety of mammalian sources. *See, e.g.*, Caetano, A.R., et al., *Equus Genome Res.* 9(12): 1239-1249 (1999) (horse), U.S. Patent Application Publication US 2002/0110871 A1 (rat, mouse, bovine, canine, porcine), U.S. Patent Application Nos. 09/344,639 and 09/231,422 (human). By way of nonlimiting example, PTH derived from the following sources and having the following peptide sequences are contemplated herein:

[0099] Human PTH_{1-84} (SEQ ID NO: 1): SER-VAL-SER-GLU-ILE-GLN-LEU-MET-HIS-ASN-LEU-GLY-LYS-HIS-LEU-ASN-SER-MET-GLU-ARG-VAL-GLU-TRP-LEU-ARG-LYS-LYS-LEU-GLN-ASP-VAL-HIS-ASN-PHE-VAL-ALA-LEU-GLY-ALA-PRO-LEU-ALA-PRO-ARG-ASP-ALA-GLY-SER-GLN-ARG-PRO-ARG-LYS-LYS-GLU-ASP-ASN-VAL-LEU-VAL-GLU-SER-HIS-GLU-LYS-SER-LEU-GLY-GLU-ALA-ASP-LYS-ALA-ASP-VAL-ASN-VAL-LEU-THR-LYS-ALA-LYS-SER-GLN.

[00100] Rat PTH_{1-84} (SEQ ID NO: 2): ALA-VAL-SER-GLU-ILE-GLN-LEU-MET-HIS-ASN-LEU-GLY-LYS-HIS-LEU-ALA-SER-VAL-GLU-ARG-MET-GLN-TRP-LEU-ARG-LYS-LYS-LEU-GLN-ASP-VAL-HIS-ASN-PHE-VAL-SER-LEU-GLY-VAL-GLN-MET-ALA-ALA-ARG-GLU-GLY-SER-TYR-GLN-ARG-PRO-THR-LYS-LYS-GLU-ASP-ASN-VAL-LEU-VAL-ASP-GLY-ASN-SER-LYS-SER-LEU-GLY-GLU-GLY-ASP-LYS-ALA-ASP-VAL-ASP-VAL-LEU-VAL-LYS-ALA-LYS-SER-GLN.

[00101] Mouse PTH_{1-84} (SEQ ID NO: 3): ALA-VAL-SER-GLU-ILE-GLN-LEU-MET-HIS-ASN-LEU-GLY-LYS-HIS-LEU-ALA-SER-VAL-GLU-ARG-MET-GLN-TRP-LEU-ARG-ARG-LYS-LEU-GLN-ASP-MET-HIS-ASN-PHE-VAL-SER-LEU-GLY-VAL-GLN-MET-ALA-ALA-ARG-ASP-GLY-SER-HIS-GLN-LYS-PRO-THR-LYS-LYS-GLU-GLU-ASN-VAL-LEU-VAL-ASP-GLY-ASN-PRO-LYS-SER-LEU-GLY-GLU-GLY-ASP-LYS-ALA-ASP-VAL-ASP-VAL-LEU-VAL-LYS-SER-LYS-SER-GLN.

[00102] Bovine PTH_{1-84} (SEQ ID NO: 4): ALA-VAL-SER-GLU-ILE-GLN-PHE-MET-HIS-ASN-LEU-GLY-LYS-HIS-LEU-SER-SER-MET-GLU-ARG-VAL-GLU-TRP-LEU-ARG-LYS-LYS-LEU-GLN-ASP-VAL-HIS-ASN-PHE-VAL-ALA-LEU-GLY-ALA-SER-ILE-ALA-TYR-ARG-ASP-GLY-SER-SER-GLN-ARG-PRO-ARG-LYS-LYS-GLU-ASP-ASN-VAL-LEU-VAL-GLU-SER-HIS-GLN-LYS-SER-LEU-GLY-GLU-ALA-ASP-LYS-ALA-ASP-VAL-ASP-VAL-LEU-ILE-LYS-ALA-LYS-PRO-GLN.

[00103] Canine PTH_{1-84} (SEQ ID NO: 5): SER-VAL-SER-GLU-ILE-GLN-PHE-MET-HIS-ASN-LEU-GLY-LYS-HIS-LEU-SER-SER-MET-GLU-ARG-VAL-GLU-TRP-LEU-ARG-LYS-LYS-LEU-GLN-ASP-VAL-HIS-ASN-PHE-VAL-ALA-LEU-GLY-ALA-PRO-ILE-ALA-HIS-ARG-ASP-GLY-SER-SER-GLN-ARG-PRO-LEU-LYS-LYS-GLU-ASP-ASN-VAL-LEU-VAL-GLU-SER-TYR-GLN-LYS-SER-LEU-GLY-GLU-ALA-ASP-LYS-ALA-ASP-VAL-ASP-VAL-LEU-THR-LYS-ALA-LYS-SER-GLN.

[00104] Porcine PTH_{1-84} (SEQ ID NO: 6): SER-VAL-SER-GLU-ILE-GLN-PHE-MET-HIS-ASN-LEU-GLY-LYS-HIS-LEU-SER-SER-LEU-GLU-ARG-VAL-GLU-TRP-LEU-ARG-LYS-LYS-LEU-GLN-ASP-VAL-HIS-ASN-PHE-VAL-ALA-LEU-GLY-ALA-SER-ILE-VAL-HIS-ARG-ASP-GLY-GLY-SER-GLN-ARG-PRO-ARG-LYS-LYS-GLU-ASP-ASN-VAL-LEU-VAL-GLU-SER-HIS-GLN-LYS-SER-LEU-GLY-GLU-ALA-ASP-LYS-ALA-ALA-VAL-ASP-VAL-LEU-ILE-LYS-ALA-LYS-PRO-GLN.

[00105] Horse PTH_{1-86} (SEQ ID NO: 7): LYS-ARG-SER-VAL-SER-GLU-ILE-GLN-LEU-MET-HIS-ASN-LEU-GLY-LYS-HIS-LEU-ASN-SER-VAL-GLU-ARG-VAL-GLU-TRP-LEU-ARG-LYS-LYS-LEU-GLN-ASP-VAL-HIS-ASN-PHE-ILE-ALA-LEU-GLY-ALA-PRO-ILE-PHE-HIS-ARG-ASP-GLY-GLY-SER-GLN-ARG-PRO-ARG-LYS-LYS-GLU-ASP-ASN-VAL-LEU-ILE-GLU-SER-HIS-GLN-XXX-SER-LEU-GLY-GLU-ALA-ASP-LYS-ALA-ASP-VAL-ASP-VAL-LEU-SER-LYS-THR-LYS-SER-GLN.

VII. EXEMPLARY MODES FOR CARRYING OUT THE INVENTION

[00106] In disclosing the present invention, one should remember that there are a number of closely analogous, species dependent forms of PTH (see above). The amino acid sequence of hPTH is shown in FIGURE 1. However, for rat PTH, bovine PTH, or porcine PTH, for example, one finds the substitutions at some of the amino acids in the hPTH sequence (*see, e.g.*, SEQ ID NOs: 1-7). For the purposes of the present invention, one can use interchangeably antibodies or antibody fragments to forms of these PTHs, although it is preferred to use an antibody with specificity for PTH having a sequence matching the species in which the PTH measurements are made.

A. WHOLE PTH IMMUNOASSAY

[00107] A preferred embodiment of the present invention is an immunoradiometric assay 15 (IRMA), often referred to as a sandwich assay, as shown FIGURES 2 and 3. Elements employed in such an assay (10) include a capture antibody (12) attached to a solid support (14) and a signal antibody (16) having a label (18), attached thereto (20). Typically, one selects a capture antibody that is specific for C-terminal PTH fragments (22), while the label antibody is specific for the initial wPTH peptide sequence which comprises a domain for

adenylate cyclase activation (24), as shown in FIGURE 2. However, one could reverse the specificity of these antibodies, as is shown in FIGURE 3.

[00108] Alternatively, one could create an immunoassay in which wPTH is either precipitated from solution or otherwise differentiated in a solution, as in conventional precipitating assays or turbidometric assays. For example, one can use at least three antibodies to form a precipitating mass. In addition to the initial wPTH sequence antibody and a C-terminal antibody, one can use at least a third antibody which attaches to the mid portion of Pm. The combined mass of wPTH and the at least three antibodies would form a labeled precipitating mass which can be measured by conventional techniques.

[00109] Another method would be to couple the initial wPTH sequence antibody to colloidal solid supports, such as latex particles. More specifically, one can create a signal antibody by iodinating 50 micrograms of affinity purified goat anti-(1-6) PTH antibody (Scantibodies Laboratory, Inc., Santee California, U.S.A.) by oxidation with chloramine T, incubation for 25 seconds at room temperature with 1 millicurie of 125-1 radioisotope and reduction with sodium metabisulfate. Unincorporated 125-1 radioisotope is separated from the 125-1-Goat anti- (1-6) PTH signal antibody by, passing the iodination mixture over a PD-1 0 desalting column (Pharmacia, Uppsala, Sweden) and following the manufacturers instructions. The fractions collected from the desalting column are measured in a gamma counter and those fractions representing the 125-1-goat anti-(1-6) PTH antibody are pooled and diluted to approximately 300,000 D PM (disintegrations per minute) per 100 microliters. This solution is the tracer solution to be used in the whole PTH IRMA.

[00110] Capture antibody coated tubes can be created by attaching affinity purified goat anti PTH 39-84 antibody, (Scantibodies Laboratory, Inc., Santee, California, U.S.A.), to 12 x 75 mm polystyrene tubes (Nunc, Denmark) by means of passive absorption techniques which are known to those of skill in the art. The tubes are emptied and dried, 20 creating solid phase antibody coated tubes.

[00111] To conduct a whole PTH assay of a sample, 200 microliter samples of human serum are added to the solid phase antibody coated tubes. To each tube is added 100 microliters of the tracer solution (labeled goat anti-(1-6) PTH signal antibody). The tubes 25 are incubated at room temperature with shaking at 170 rpm for 20-22 hours. During this time the immunochemical reaction of forming the sandwich of {solid phase goat anti-(39- 84) PTH antibody} --{ whole PTH} --{ 125-1-goat anti-(1-6) PTH antibody} takes place. Following this incubation, the test tubes are washed with distilled water. Radioactivity on the solid phase, which amount corresponds to the quantity of wPTH present, is measured using a gamma counter. The radioactivity data for the samples is processed by conventional analysis with use

of the results from standards and controls and computer software in order that the concentration of whole PTH in the samples may be ascertained. FIGURE 4 shows a standard curve for such an assay.

B. INITIAL WHOLE PTH SEQUENCE PEPTIDE

[00112] In order to make the signal antibody in the above assays, first one makes a synthetic PTH peptide corresponding either to human PTH_{1-8}, rat PTH_{1-8}, mouse PTH_{1-8}, bovine PTH_{1-8}, canine PTH_{1-8}, porcine PTH_{1-8}, horse PTH_{1-8}, human PTH_{1-15}, rat PTH_{1-15}, mouse PTH_{1-15}, bovine PTH_{1-15}, canine PTH_{1-15}, porcine PTH_{1-15}, or horse PTH_{1-15}, or at least four amino acids in the common sequence. Although not bound by theory, suitable synthetic PTH peptides may extend beyond the PTH_{1-8} as illustrated above, i.e., to position 84 of PTH_{1-84}. For example, any of the variety of PTH N-terminal fragments are suitable initial peptide sequences for this aspect. The selected peptide can play two roles in making an assay, first as a specific source for creating a polyclonal antibody or monoclonal antibody source for signal antibody or capture antibody, and second as part of an affinity purification means for isolating the desired signal antibody or capture antibody.

[00113] Briefly, such a peptide can be synthesized on an Applied Biosystems, Inc. (Foster City, California, U.S.A.) Model 431 automated peptide synthesizer employing Fmoc (9-fluoronylmethoxycarbonyl) as the alpha-amino protecting group. All amino acids and solvents are from Applied Biosystems and are of synthesis grade. Following synthesis, the peptide is cleaved from the resin, and side chains are de-blocked, using a cleavage cocktail 20 containing 6.67% phenol, 4.4% (v/v) thioanisole and 8.8% ethanedithiol in trifluoroacetic acid (TFA). The cleaved peptide is precipitated and washed several times in cold diethyl ether. It is then dissolved in water and lyophilized. The crude peptide is subjected to amino acid analysis (Waters PICO-TAG System, Boston, Massachusetts, U.S.A.) and reversed-phase HPLC using a VYDAC (TM) C8 column with 0.1% TFA in water and 25 99.9% acetonitrile in 0.1% TF A as the mobile buffers. The presence of a single major peak along with the appropriate amino acid composition is taken as evidence that the peptide is suitable for further use.

[00114] The resulting peptide is then attached to cross linked agarose beads (activated Sepharose 4B from Pharmacia, Uppsala, Sweden) according to instructions from the manufacturer. Armed with the initial peptide sequence on a bead, one can affinity purify a polyclonal antibody serum source to isolate the initial sequence antibody for the wPTH immunoassay.

[00115] In a particularly preferred embodiment, initial sequence PTH antibodies successfully distinguish between initial PTH peptides and C-terminal and mid-terminal PTH peptides, such that they specifically bind initial sequence PTH peptides.

[00116] In another preferred embodiment, the above methods are utilized to synthesize mid-terminal and C-terminal PTH peptide fragments. After synthesis of these peptides, the above methods are utilized to generate and isolate mid-terminal PTH antibodies and/or C-terminal PTH antibodies. In a preferred embodiment, mid-terminal PTH antibodies specifically bind mid-terminal PTH peptide fragments. In another preferred embodiment, C-terminal PTH antibodies specifically bind C-terminal PTH peptide fragments.

C. INITIAL SEQUENCE WHOLE PTH ANTIBODY

[00117] In another embodiment, to create an affinity-purified initial sequence whole PTH antibody, one first uses a PTH sequence peptide having an intact N-terminal as described above as part of an immunogen for injection into a goat.. For example, a PTH peptide ranging from PTH_{1-34} to PTH_{1-84} may be utilized. The peptide can be used either by itself as an injectible immunogen, incorporated into a non PTH peptide having a molecular weight, typically, of between about 5,000 and 10,000,000, or as part of the wPTH complete sequence. The immunogen is mixed with an equal volume of Freund's complete adjuvant which is a mixture of light mineral oil, Arlacel detergent, and inactivated mycobacterium tuberculosis bacilli. The resulting mixture is homogenized to produce an aqueous/oil emulsion which is injected into the animal (typically a goat) for the primary immunization. The immunogen dose is approximately 50-400 micrograms. The goats are injected monthly with the same dose of immunogen complex except no mycobacterium tuberculosis bacilli is used in these subsequent injections. The goats are bled monthly, approximately three months after the 20 primary immunization. The serum (or antiserum) is derived from each bleeding by separating the red blood cells from the blood by centrifugation and removing the antiserum which is rich in initial sequence PTH antibodies.

[00118] To purify the antiserum for the desired initial sequence whole PTH antibody, one packs a separation column with a PTH sequence peptide (e.g., a PTH peptide ranging from PTH_{1-5} to PTH_{1-15}) bound beads described above, washes the column and equilibrates it with 0.01 M phosphate buffered saline (PBS). The antiserum is loaded onto the column and washed with 0.01 M PBS in order to remove antibodies without the initial sequence PTH specificity. The bound specific goat initial sequence PTH polyclonal antibody is eluted from the solid phase that includes optionally PTH_{1-5} to PTH_{1-15} in the column by passing an elution solution of 0.1 M glycine hydrochloride buffer, pH 2.5 through the column. The eluted polyclonal antibody is neutralized after it leaves the column with either the addition of 1.0 M phosphate buffer, pH 7.5 or by a buffer exchange with 0. 01 M PBS, as is known to those of skill in the art. The polyclonal antibody is stored at 2-8 degrees centigrade.

[00119] In another embodiment, to create an affinity-purified anti-(1-6) PTH antibody, one first uses a selected initial PTH sequence peptide as described above as part of an immunogen for injection into a goat. The peptide can be used either by itself as an injectible immunogen, incorporated into a non PTH peptide having a molecular weight, typically, of between about 5,000 and 10,000,000, or as part of the wPTH complete sequence. The immunogen is mixed with an equal volume of Freund's complete adjuvant which is a mixture of light mineral oil, Arlacel detergent, and inactivated mycobacterium tuberculosis bacilli. The resulting mixture is homogenized to produce an aqueous/oil emulsion which is injected into the animal (typically a goat) for the primary immunization. The immunogen dose is approximately 50-400 micrograms. The goats are injected monthly with the same dose of immunogen complex except no mycobacterium tuberculosis bacilli is used in these subsequent injections. The goats are bled monthly, approximately three months after the 20 primary immunization. The serum (or antiserum) is derived from each bleeding by separating the red blood cells from the blood by centrifugation and removing the antiserum which is rich in (1-6) PTH antibodies.

[00120] To purify the antiserum for the desired (1-6) PTH antibody, one packs a separation column with the initial PTH sequence peptide bound beads described above, washes the column and equilibrates it with 0.01 M phosphate buffered saline (PBS). The antiserum is loaded onto the column and washed with 0.01 M PBS in order to remove antibodies without the (1-6) PTH specificity. The bound specific goat anti-(1-6) PTH polyclonal antibody is eluted from the solid phase PTH 1-6 in the column by passing an elution solution of 0.1 M glycine hydrochloride buffer, pH 2.5 through the column. The eluted polyclonal antibody is neutralized after it leaves the column with either the addition of 1.0 M phosphate buffer, pH 7.5 or by a buffer exchange with 0. 01 M PBS, as is known to those of skill in the art. The polyclonal antibody is stored at 2-8 degrees centigrade.

[00121] One of skill in the art would understand that there are acceptable variations in the above practices. See, e.g., Harlow E, Lane D: Antibodies: A laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1988; Kohler & Milstein, Nature, 256: 495-7 (1975). While not bound by theory, the above practices are suitable for production of other PTH N-terminal antibodies using selected N-terminal PTH sequence peptides as described herein.

[00122] In a particularly preferred embodiment, initial sequence PTH antibodies successfully distinguish between initial PTH peptides and both C-terminal and mid-terminus PTH peptides, such that they specifically bind initial sequence PTH peptides.

D. C-TERMINAL SEQUENCE PTH ANTIBODY

[00123] To create an affinity-purified anti-(60-84) PTH antibody, one first uses a selected C-terminal PTH sequence peptide as described above as part of an immunogen for injection into a goat. In another embodiment, the immunogen comprises whole PTH peptide, *e.g.*, PTH_{1-84}. The peptide can be used either by itself as an injectible immunogen, incorporated into a non PTH peptide having a molecular weight, typically, of between about 5,000 and 10,000,000. The immunogen is mixed with an equal volume of Freunds complete adjuvant which is a mixture of light mineral oil, Arlacel detergent, and inactivated mycobacterium tuberculosis bacilli. The resulting mixture is homogenized to produce an aqueous/oil emulsion which is injected into the animal (typically a goat) for the primary immunization. The immunogen dose is approximately 50-400 micrograms. The goats are injected monthly with the same dose of immunogen complex except no mycobacterium tuberculosis bacilli is used in these subsequent injections. The goats are bled monthly, approximately three months after the 20 primary immunization. The serum (or antiserum) is derived from each bleeding by separating the red blood cells from the blood by centrifugation and removing the antiserum which contains (60-84) PTH antibodies. In another embodiment, the antiserum is removed which contains (60-84) PTH antibodies in addition to other PTH antibodies, e.g., whole PTH antibodies.

[00124] To purify the antiserum for the desired (60-84) PTH antibody, one packs a separation column with the C-terminal PTH sequence peptide bound beads described above, washes the column and equilibrates it with 0.01 M phosphate buffered saline (PBS). The antiserum is loaded onto the column and washed with 0.01 M PBS in order to remove antibodies without the (60-84) PTH specificity. The bound specific goat anti-(60-84) PTH polyclonal antibody is eluted from the solid phase PTH 1-6 in the column by passing an elution solution of 0.1 M glycine hydrochloride buffer, pH 2.5 through the column. The eluted polyclonal antibody is neutralized after it leaves the column with either the addition of 1.0 M phosphate buffer, pH 7.5 or by a buffer exchange with 0. 01 M PBS, as is known to those of skill in the art. The polyclonal antibody is stored at 2-8 degrees centigrade.

[00125] One of skill in the art would understand that there are acceptable variations in the above practices. See, e.g., Harlow E, Lane D: Antibodies: A laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1988; Kohler & Milstein, Nature, 256: 495-7 (1975). While not bound by theory, the above practices are suitable for production of other PTH C-terminal antibodies using selected C-terminal PTH sequence peptides as described herein. For example, PTH peptides such as PTH_{53-84}, PTH_{60-84}, PTH_{64-84}, PTH_{65-84}, PTH_{39-84}, PTH_{23-84}, PTH_{19-84}, and other C-terminal PTH peptides as described above.

[00126] In a particularly preferred embodiment, C-terminal PTH antibodies successfully distinguish between C-terminal peptides and both initial sequence and mid-terminus PTH peptides, such that they specifically bind C-terminal PTH peptides.

E. MID-TERMINUS SEQUENCE PTH ANTIBODY

[00127] To create an affinity-purified anti-(44-68) PTH antibody, one first uses a selected mid-terminus PTH sequence peptide as described above as part of an immunogen for injection into a goat. In another embodiment, the immunogen comprises whole PTH peptide, *e.g.*, PTH_{1-84}. The peptide can be used either by itself as an injectible immunogen, incorporated into a non PTH peptide having a molecular weight, typically, of between about 5,000 and 10,000,000. The immunogen is mixed with an equal volume of Freunds complete adjuvant which is a mixture of light mineral oil, Arlacel detergent, and inactivated mycobacterium tuberculosis bacilli. The resulting mixture is homogenized to produce an aqueous/oil emulsion which is injected into the animal (typically a goat) for the primary immunization. The immunogen dose is approximately 50-400 micrograms. The goats are injected monthly with the same dose of immunogen complex except no mycobacterium tuberculosis bacilli is used in these subsequent injections. The goats are bled monthly, approximately three months after the 20 primary immunization. The serum (or antiserum) is derived from each bleeding by separating the red blood cells from the blood by centrifugation and removing the antiserum which contains (44-68) PTH antibodies. In another embodiment, the antiserum is removed which contains (44-68) PTH antibodies in addition to other PTH antibodies, e.g., whole PTH antibodies.

[00128] To purify the antiserum for the desired (44-68) PTH antibody, one packs a separation column with the mid-terminus PTH sequence peptide bound beads described above, washes the column and equilibrates it with 0.01 M phosphate buffered saline (PBS). The antiserum is loaded onto the column and washed with 0.01 M PBS in order to remove antibodies without the (44-68) PTH specificity. The bound specific goat anti-(44-68) PTH polyclonal antibody is eluted from the solid phase PTH 1-6 in the column by passing an elution solution of 0.1 M glycine hydrochloride buffer, pH 2.5 through the column. The eluted polyclonal antibody is neutralized after it leaves the column with either the addition of 1.0 M phosphate buffer, pH 7.5 or by a buffer exchange with 0. 01 M PBS, as is known to those of skill in the art. The polyclonal antibody is stored at 2-8 degrees centigrade.

[00129] One of skill in the art would understand that there are acceptable variations in the above practices. See, e.g., Harlow E, Lane D: Antibodies: A laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1988; Kohler & Milstein, Nature, 256: 495-7 (1975). While not bound by theory, the above practices are suitable for production

of other PTH mid-terminus antibodies using selected mid-terminus PTH sequence peptides as described herein. For example, PTH peptides such as PTH_{44-60}, PTH_{7-53}, PTH_{12-53}, PTH_{17-53}, PTH_{22-53}, PTH_{27-53}, PTH_{30-35}, PTH_{32-53}, PTH_{32-53}, PTH_{37-53}, PTH_{42-53}, PTH_{47-53}, and other mid-terminal PTH peptides as described above.

[00130] In a particularly preferred embodiment, mid-terminus PTH antibodies successfully distinguish between mid-terminus peptides and both initial sequence and C-terminal PTH peptides, such that they specifically bind mid-terminus PTH peptides.

F. COMPARISON BETWEEN SECOND GENERATION WHOLE PTH AND TOTAL PTH ASSAYS

[00131] The present whole IRMA assay was compared to a conventional intact PTH or I-PTH immunoassay, the Allegro Nichols Intact-PTH assay, (which is commercially available and made by Nichols Institute Diagnostics of San Juan Capistrano, California, U.S.A.), in both PTH normal persons and those suffering from chronic uremia. This I- PTH immunoassay, detects both PTH_{7-84} and wPTH (see FIGURE 10).

[00132] FIGURE 5 shows the results for 34 normal human serum samples from healthy subjects which were assayed both by the present wPTH IRMA and the above I-PTH assay. In every case, the level of wpm detected by the IRMA is lower than that reported by the I-PTH assay, demonstrating the ability of the present IRMA to avoid detecting the interfering large, non (1-84) PTH fragment detected by the I-pm assay, (see FIGURE 11). FIGURE 6 illustrates how such interference can occur. An N-terminal PTH specific signal antibody which is not specific to the initial PTH peptide sequence, as in the present invention, can detect not only wPTH (as in the upper part of FIGURE 6), but also can detect PIN, the large, non (1-84) PTH fragment, (as in the lower part of FIGURE 6).

[00133] A comparison of assay results for 157 chronic uremic patients is shown in FIGURE 7. Serum samples from these patients were measured using the wPTH IRMA and the above I-PTH assay. In every case the wPTH levels are lower than I-PTH values.

G. CYCLASE ACTIVE PTH/PTH FRAGMENT RATIO ASSAY

[00134] Another preferred embodiment of the present invention is an immunoradiometric assay (IRMA), often referred to as a sandwich assay. As described herein, elements employed in such an assay include a capture antibody attached to a solid support and a signal antibody having a label, attached thereto. Typically, one selects a capture antibody that is specific for C-terminal PTH fragments, while the label antibody is specific for a mid-terminus PTH peptide sequence as described herein. However, one could reverse the specificity of these antibodies.

[00135] Alternatively, one could create an immunoassay in which PTH fragments are either precipitated from solution or otherwise differentiated in a solution, as in conventional precipitating assays or turbidometric assays. For example, one can use at least two or more antibodies to form a precipitating mass. These antibodies having specificity for N-terminal, C-terminal and/or mid-terminal portions of PTH. The combined mass of the PTH fragment and the antibodies would form a labeled precipitating mass which can be measured by conventional techniques.

[00136] Another method would be to couple C-terminal PTH sequence antibody to colloidal solid supports, such as latex particles. In addition, a signal antibody can be created by iodinating 50 micrograms of affinity purified goat anti-(7-53) PTH antibody by oxidation with chloramine T, incubation for 25 seconds at room temperature with 1 millicurie of 125-1 radioisotope and reduction with sodium metabisulfate. Unincorporated 125-1 radioisotope is separated from the 125-1-Goat anti- (7-53) PTH signal antibody by, passing the iodination mixture over a PD-l 0 desalting column (Pharmacia, Uppsala, Sweden) and following the manufacturers instructions. The fractions collected from the desalting column are measured in a gamma counter and those fractions representing the 125-1-goat anti-(7-53) PTH antibody are pooled and diluted to approximately 300,000 D PM (disintegrations per minute) per 100 microliters. This solution is the tracer solution to be used in the PTH assay.

[00137] Other signal antibodies can also be provided using affinity purified goat anti-(12-53) PTH antibody, purified goat anti-(17-53) PTH antibody, purified goat anti-(22-53) PTH antibody, purified goat anti-(27-53) PTH antibody, purified goat anti-(32-53) PTH antibody, purified goat anti-(37-53) PTH antibody, purified goat anti-(42-53) PTH antibody, purified goat anti-(47-53) PTH antibody, and the like. Antibodies specific for other mid-terminus PTH peptide fragments and epitopes described herein are also contemplated. Optimally, PTH antibodies specific for different PTH portions are obtained utilizing different goats and ELISA methods may be utilized to determine optimum antibody generation and use. See, e.g., Harlow E, Lane D: Antibodies: A laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1988.

[00138] Capture antibody coated tubes can be created by attaching affinity purified goat anti-(39-84) PTH antibody to 12 x 75 mm polystyrene tubes (Nunc, Denmark) by means of passive absorption techniques which are known to those of skill in the art. The tubes are emptied and dried, 20 creating solid phase antibody coated tubes. The present methods are also useful to create solid phase antibody coated tubes having any of a variety of C-terminal PTH antibodies. For example, antibodies generated specific for PTH_{40-84} ranging to those specific

for PTH_{60-84} and PTH_{65-84} are useful in the present methods. One of skill in the art would understand that the specificity for the capture and label/signal antibodies may be reversed.

[00139] A selection of these specific antibodies may be utilized to assay samples from patients having adynamic low bone turnover. In one example, a sample of 50-100 ml blood is drawn from a subject having adynamic low bone turnover. The sample is then subject to multiple sandwich assays utilizing the capture and label antibodies described herein above. Each assay determines the level of whole PTH, in addition to one or more PTH fragments present in the sample. The level of whole PTH is then compared with a variety of permutations of PTH fragment levels and total PTH levels. The comparison results are then viewed in light of corresponding bone histological data for the patient to determine one or more comparison schemes that are predictive of bone turnover rate. Ratio results that are produced through the practice of this assay are represented in Table 1 and are further described hereinbefore.

[00140] A PTH ratio is predictive of certain bone turnover rates in a subject. The present disclosure recognizes that additional PTH fragments may be present in a sample beyond PTH_{7-84}, and often effect the bone turnover rate. Thus, the present compositions, kits and methods are particularly useful to account for PTH fragments in addition to PTH_{7-84}, having associated therapeutic and predictive benefits for a subject. Although not bound by theory, one component of this discovery is illustrated, in part, by the observation that although a percentage of circulating large PTH fragments are inactive, others exhibit antagonistic effects to those exhibited by whole PTH. Thus, accounting for all, or a majority of, the circulating PTH fragments in a particular assay will reduce the variability of the result, and increase its therapeutic, diagnostic and predictive potential.

H. CLINICAL USE

[00141] The present wPTH and PIN assays have been used in a clinical setting involving 188 persons. The group included 31 persons having normal healthy parathyroid glands and 157 patients with chronic uremia who are undergoing dialysis on a continuous basis. Each person had a blood sample drawn which was assayed using a wPTH assay from Scantibodies Laboratory, Inc. as well as an I-PTH assay from Nichols Institute which gave total PTH values.

[00142] Table 3 shows the results individually and comparatively, of the wPTH, PIN, and 10 total PTH assays from chronic uremic patients on dialysis.

TABLE 3

Patient No.	*Total PTH pg/ml*	*CAP pg/ml*	*CIP pg/ml*	*CIP to Total PTH*	*CIP to CAP*	*CAP to Total PTH*
1	1410	740	670	48%	91%	52%
2	185	89	96	52%	108%	48%
3	231	104	127	55%	122%	45%
4	1020	590	430	42%	73%	53%
5	270	159	111	41%	70%	59%
6	201	100	101	50%	101%	50%
7	380	100	280	74%	280%	26%
8	460	277	183	40%	66%	60%
9	380	197	183	48%	93%	52%
10	880	522	358	41%	69%	59%
11	310	154	156	50%	101%	50%
12	880	451	429	49%	95%	51%
13	670	418	252	38%	60%	63%
14	390	221	169	43%	76%	57%
15	170	108	62	36%	57%	64%
16	510	381	129	25%	34%	75%
17	200	67	133	67%	199%	34%
18	170	109	61	36%	56%	64%
19	360	199	161	45%	81%	55%
20	260	164	96	37%	59%	63%
21	440	372	68	15%	18%	85%
22	120	51.7	68.3	57%	132%	43%
23	600	527	73	12%	14%	83%
24	220	130	90	41%	69%	59%
25	190	136	54	28%	40%	72%
26	220	118	102	46%	86%	54%
27	630	334	296	47%	89%	53%
28	150	90	60	40%	67%	60%
29	170	106	64	38%	60%	62%
30	810	489	321	40%	66%	60%
31	570	319	251	44%	79%	56%
32	570	133	103	18%	22%	82%
33	400	564	100	25%	33%	75%

Patient No.	*Total PTH pg/ml*	*CAP pg/ml*	*CIP pg/ml*	*CIP to Total PTH*	*CIP to CAP*	*CAP to Total PTH*
34	560	89	182	33%	48%	68%
35	310	121	189	61%	156%	39%
36	240	98	142	59%	145%	41%
37	280	133	157	54%	118%	48%
38	230	124	106	46%	85%	54%
39	350	319	31	9%	10%	91%
40	200	133	67	34%	50%	67%
41	920	564	356	39%	63%	61%
42	210	89	121	58%	136%	42%
43	1990	904	1086	55%	120%	45%
44	300	212	88	29%	42%	71%
45	260	132	128	49%	97%	51%
46	140	72	68	49%	94%	51%
47	250	129	121	48%	94%	52%
48	130	72	58	45%	81%	56%
49	1840	1000	840	46%	84%	54%
50	280	167	113	40%	68%	60%
51	490	268	222	45%	83%	55%
52	150	77.1	72.9	49%	95%	51%
53	140	58.1	81.9	59%	141%	42%
54	210	92.7	117.3	56%	127%	44%
55	160	79	81	51%	103%	49%
56	480	296	184	38%	62%	62%
57	480	281	199	41%	71%	59%
58	270	120	150	56%	125%	44%
59	97	45	52	54%	116%	46%
60	330	154	176	53%	114%	47%
61	110	56	54	49%	96%	51%
62	660	456	204	31%	45%	69%
633	300	137	163	54%	119%	46%
64	240	145	95	40%	66%	60%
65	100	66.5	33.5	34%	50%	67%
66	410	416.3	6.3	2%	2%	102%

Patient No.	*Total PTH pg/ml*	*CAP pg/ml*	*CIP pg/ml*	*CIP to Total PTH*	*CIP to CAP*	*CAP to Total PTH*
67	410	235.7	174.3	43%	74%	57%
68	45	14.4	30.6	68%	213%	32%
69	200	102.3	97.7	49%	96%	51%
70	300	134	166	55%	124%	45%
71	320	202	118	37%	58%	63%
72	440	254	186	42%	73%	58%
73	190	99.6	90.4	48%	91%	52%
74	160	74.6	85.4	53%	114%	47%
75	600	429.8	170.2	28%	40%	72%
76	1140	632	508	45%	80%	55%
77	40	211	229	52%	109%	48%
78	450	276	174	39%	63%	61%
79	510	344	166	33%	48%	67%
80	190	62.8	127.2	67%	203%	33%
81	170	86	84	49%	98%	51%
82	180	103.4	76.6	43%	74%	57%
83	78	22.7	55.3	71%	244%	29%
84	230	117	113	49%	97%	51%
85	160	96	64	40%	67%	60%
86	220	89	131	60%	147%	40%
87	470	321.5	148.5	32%	46%	68%
88	310	137	173	56%	126%	44%
89	2050	1127	923	45%	82%	55%
90	930	414	516	55%	125%	45%
91	180	65	115	64%	177%	36%
92	560	238	322	58%	135%	43%
93	640	597	43	7%	7%	93%
94	590	382	208	35%	54%	65%
95	270	103	167	62%	162%	38%
96	560	349	211	38%	60%	62%
97	180	78	102	57%	131%	43%
98	790	429	361	46%	84%	54%
99	670	372	298	44%	80%	56%

Patient No.	*Total PTH pg/ml*	*CAP pg/ml*	*CIP pg/ml*	*CIP to Total PTH*	*CIP to CAP*	*CAP to Total PTH*
100	140	20.4	119.6	85%	586%	15%
101	190	117	73	38%	62%	62%
102	190	108	82	43%	76%	57%
103	430	217	213	50%	98%	50%
104	560	439	121	22%	28%	78%
105	500	357.7	142.3	28%	40%	72%
106	1560	777	783	50%	101%	50%
107	62	24.3	37.7	61%	155%	39%
108	430	226	204	47%	90%	53%
109	160	67.2	92.8	58%	138%	42%
110	530	346	184	35%	53%	65%
111	260	142	118	45%	83%	55%
112	580	163	41	72%	256%	28%
113	440	579	139	32%	24%	132%
114	500	232.3	267.7	54%	115%	46%
115	160	60	100	63%	167%	38%
116	340	202	138	41%	68%	59%
117	260	138	122	47%	88%	53%
118	260	119	141	54%	118%	46%
119	160	84	76	48%	90%	53%
120	130	46	84	65%	183%	35%
121	190	104	86	45%	83%	55%
122	420	334	86	20%	26%	80%
123	630	440	190	30%	43%	70%
124	75	26.4	48.6	65%	184%	35%
125	260	143	117	45%	82%	55%
126	640	409	231	36%	56%	64%
127	130	66.7	63.3	49%	95%	51%
128	700	381	319	46%	84%	54%
129	560	376	184	33%	49%	67%
130	240	107	133	55%	124%	45%
131	110	63	47	43%	75%	57%
132	420	297	123	29%	41%	71%

Patient No.	*Total PTH pg/ml*	*CAP pg/ml*	*CIP pg/ml*	*CIP to Total PTH*	*CIP to CAP*	*CAP to Total PTH*
133	580	229	351	61%	153%	39%
134	310	201.2	108.8	35%	54%	65%
135	160	97.9	62.1	39%	63%	61%
136	290	138.7	151.3	52%	109%	48%
137	200	96.2	103.8	52%	108%	48%
138	770	662.7	107.3	14%	16%	86%
139	290	130.7	159.3	55%	122%	45%
140	260	219	41	16%	19%	84%
141	350	211	139	40%	66%	60%
142	730	463.5	266.5	37%	57%	63%
143	490	231	259	53%	112%	47%
144	160	87	73	46%	84%	54%
145	380	222	158	42%	71%	58%
146	210	93.5	116.5	55%	125%	45%
147	630	383.4	246.6	39%	64%	61%
148	150	83.2	66.8	45%	80%	55%
149	320	152.5	167.5	52%	110%	48%
150	900	467.6	432.4	48%	92%	52%
151	1180	818.6	361.4	31%	44%	69%
152	120	38.4	81.6	68%	213%	32%
153	5230	1388	3842	73%	277%	27%
154	34	10.5	23.5	69%	224%	31%
155	1020	590.6	429.4	42%	73%	58%
156	280	76.6	103.4	57%	135%	43%
157	120	51.1	68.9	57%	135%	43%
Median	300	154	127	46%	84%	54%

[00143] TABLE 4 shows the results, individually and comparatively, of the wPTH, PIN, and total PTH assays from the normals.

TABLE 4

Patient No.	*Total PTH pg/ml*	*CAP pg/ml*	*CIP pg/ml*	*CIP to Total PTH*	*CIP to CAP*	*CAP to Total PTH*
1	17.13	3.32	13.81	81%	416%	19%
2	32.92	10.49	22.43	68%	214%	32%
3	31.32	10.31	21.01	67%	204%	33%
4	41.84	12.72	29.12	70%	229%	30%
5	33.03	10.09	22.94	69%	227%	31%
6	44.32	14.23	30.09	68%	211%	32%
7	31.47	6.80	24.67	78%	363%	22%
8	20.82	10.03	10.79	52%	108%	48%
9	34.64	15.95	18.69	54%	117%	46%
10	23.69	5.25	18.44	78%	351%	22%
11	53.98	17.82	36.16	67%	203%	33%
12	52.71	18.83	33.88	64%	180%	36%
13	26.92	5.63	21.29	79%	378%	21%
14	39.93	11.86	28.07	70%	237%	30%
115	48.84	20.47	28.37	58%	139%	42%
16	29.56	13.68	15.88	54%	116%	46%
17	36.19	14.69	21.50	59%	146%	41%
18	20.96	6.99	13.97	67%	200%	33%
19	59.29	27.89	31.40	53%	113%	47%
20	45.57	18.23	27.34	60%	150%	40%
21	35.64	18.72	16.92	47%	90%	53%
22	38.53	19.56	18.97	49%	97%	51%
23	21.71	9.34	12.37	57%	132%	43%
24	32.42	13.51	18.91	58%	140%	42%
25	28.50	10.41	18.09	63%	174%	37%
26	18.27	7.80	10.37	57%	133%	43%
27	39.96	17.29	22.67	57%	131%	43%
28	34.08	15.24	18.84	55%	124%	45%
29	42.95	19.59	23.36	54%	119%	46%

Patient No.	*Total PTH pg/ml*	*CAP pg/ml*	*CIP pg/ml*	*CIP to Total PTH*	*CIP to CAP*	*CAP to Total PTH*
30	38.40	12.16	26.24	68%	216%	32%
31	47.57	18.45	29.12	61%	158%	39%
MEDIAN	34.64	13.51	21.50	61%	158%	39%

[00144] Clearly, the statistically significant differences in the medians of these two groups demonstrates that one can differentiate between the two by using these assays alone or by comparing their respective values.

TABLE 5

Sample Type	Total PTH *pg/ml Median*	*CAP pg/ml Median*	*CIP pg/ml Median*	*CIP to Total PTH Median*	*CIP to CAP Median*	*CAP to Total PTH Median*
Chronic uremia (n=157)	300	154	127	46%	84%	55%
Normal (n=31)	34.64	13.51	21.50	61%	158%	37%
P-Value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

I. CHARACTERIZATION OF THE "WHOLE" PTH IRMA ASSAY

[00145] This new Whole PTH two-site assay (Scantibodies Laboratories, Santee, CA, USA) first employees and antibody that recognizes the 39-84 region of the PTH molecule. This antibody, produced in a goat and affinity purified, is present in relative excess and is immobilized onto polystyrene-coated tubes. The second antibody, also developed in a goat, was also affinity purified and recognizes only the first six amino acids (1 to 6; Ser-Val-Ser-Glu-Ile-Gln) of the human PTH molecule (Fig. 1). This anti-hPTH assay uses synthetic human PTH 1-84 as the standard, with a limit of detection of approximately 1 to 2 pg/mL. Normal values range from 5 to 35 pg/mL. The interassay and intra-assay coefficients of variation were found to be between 2 and 7% and recovery was from 96 to 106%. The Whole PTH assay was

compared with the Intact PTH assay purchases from the Nichols Institute (I-Nichols, San Jan Capistrano, CA, USA). Synthetic human PTH 1-84 and 7-84 were purchased from Bachem (Torrance, CA USA). To assess circulating levels of hPTH 1-84 and non-(1-84) PTH, heparinized blood samples were obtained before dialysis from 28 patients who had been maintained on chronic hemodialysis for 1.2 to 7.5 years and from 14 renal transplant patients (1 to 6 years).

1. STUDIES *IN VITRO*

[00146] *Osteoblastic cell line.* To compare the biological effects of the two peptides (HPTH 1-84 and 7-84), intracellular cAMP production was measured in the rate osteosarcoma cell line ROS/17.2, which has an osteoblastic phenotype and is known to increase cAMP production in response to PTH. Cells were cultured in Ham's F12 media containing 10% fetal bovine serum. Cells were plated out in 12-well plates at a density of 30,000 cells per well and grown to confluence. Cells were washed three times with KHMS buffer at 37°C (KCl 4.0 mmol/L, $CaCl_2$ 1.25 mmol/L, $MgSO_4$ 1.25 mmol/L, KH_2PO_4 1.2 mmol/L, HEPES 10 mmol/L, NaCl 100 mmol/L, $NaHCO_3$ 37 mmol/L, and glucose 10 mmol/L, pH 7.5). cAMP production was measured using 500 µL of KHMS buffer (37°) containing isobutyl-1 methylxantine (IBMX) 1.0 mmol/L and various concentrations (10^{-11} to 10^{-8} mol/L) of hPTH 1-84 or hPTH 7-84. After a five-minute incubation, 100 µL of 1.8 mol/L pechloric acid were added. After an additional five-minute incubation at room temperature, 100 µL of 3 mol/$KHCO_3$ were added to neutralize the acid. Samples were centrifuged at 3000 rpm for 15 minutes, and the supernatants were assayed for cAMP [26].

[00147] *Analysis of PTH in human parathyroid glands.* Human parathyroid glands were placed in ice-cold phosphate-buffered saline and processed within 30 minutes of parathyroidectomy. Aliquots of parathyroid tissue were dissected, weighed, and homogenized in 500 µL of a buffer containing 100 mmol/L Tris-HCl, pH 7.5, 100 mmol/L NaCl, 1 mol/L DL-dithiothreitol, and a complete TM protease inhibitor cocktail (Boehringer-Mannheim, Mannheim, Germany). Homogenates were sonicated three times for 30 seconds each at 0°C and centrifuged at 10,000 X *g* for 15 minutes. Supernatants were kept at -70°C until measurements of 1-84 PTH, non-(1-84) PTH, and total protein were performed.

2. STUDIES *IN VIVO*

[00148] *Calcemic response.* Normal female Sprague-Dawley rats weighing 225 to 250 g (Harlan, Indianapolis, IN, USA) were parathyroidectomized (PTX) and fed a 0.02% calcium diet. Rats with a plasma calcium below 7.0 mg/dL after overnight fasting were included in the study. A 20µg dose of hPTH 1-84 or 7-84 was given intraperitoneally to PTX rats in four doses of 5 µg each at 30-minute intervals (0, 30, 60, and 90 minutes). For control

studies, the rats received vehicle (saline solution) alone. Blood was drawn via the tail at 0, 60, 90, and 120 minutes. For competition experiments, rats received an injection of hPTH 7-84 10 minutes prior to each injection of hPTH 1-84. The molar ratio of hPTH 7-84 / hPTH 1-84 was 1:1.

[00149] *Phosphaturic response.* Normal female Sprague-Dawley rats weighing 225 to 250 g were prepared for clearance studies under light anesthesia. Polyethylene catheters (PE50) were placed in the femoeral artery for the collection of blood and measurement of blood pressure (Blood Pressure Analyzer; Micro-Medic, Inc., Louisville, KY, USA), in the femoral vein for infusion and in the bladder for the collection of urine. Rats were placed in Plexiglas® holders and allowed to recover from the effect of the anesthetic for one hour. A priming dose (0.6 mL) of chemical inulin in saline was administered over a period of three minutes to achieve a plasma inulin level between 50 and 100 mg/mL. A solution of saline containing inulin to maintain this level and calcium gluconate to deliver 0.5 mg. calcium was infused at the rate of 0.03 mL/min. After equilibration, a total of four 30-minute urine collections was obtained.

[00150] To assess the effect of hPTH 1-84 on phosphate excretion, urine was collected during two control periods, after which rats received a priming bolus of 1.8 μg of hPTH 1-84 followed by a sustained infusion that delivered a total of 8.2 μg of I-PTH. After an equilibration period of 20 minutes, two 30-minute urine collections were obtained. In competition experiments, hPTH 7-84 was given five minutes prior to hPTH 1-84 at a molar ratio of 4:1.

[00151] Blood samples and blood pressure measurements were recorded at the beginning and end of the baseline period, at the beginning of the PTH infusion period, and at the end of the study. The concentration of inulin in plasma and urine was determined by the method of Führ, Kaczmarczyk, and Kruttgen, *Klin Wochenschr*, 33:729-730 (1955). The estimation of the glomerular filtration rate (GFR) by inulin clearance and the calculation of the fractional urinary excretion rate of phosphorus (FE_{P04}) were performed in the standard fashion. Blood samples were centrifuged, and plasma phosphorus and calcium concentrations were measured.

3. SERUM CHEMISTRIES

[00152] Total plasma calcium levels were determined using an atomic absorption spectrophotometer (model 1100B; Perkin Elmer, Norwalk, CT, USA). Plasma phosphorus levels were determined using an autoanalyzer (COBAS MIRA Plus; Roche, Newark, NJ, USA).

4. STATISTICAL ANALYSIS

[00153] Results are expressed as mean ± SEM. *N* indicates the number of samples. The paired *t*-test was employed to examine statistical significance, unless otherwise indicated in the text.

J. SPECIFICITY OF IRMA ASSAYS FOR hPTH 1-84

[00154] Initial studies compared the ability of the Nichols Intact (I-Nichols) PTH assay and the new Whole PTH assay to discriminate between the hPTH 1-84 and hPTH 7-84 molecules. Figure 13 shows that the Nichols "intact" PTH assay did not discriminate between human PTH 1-84 and 7-84. However, as depicted in Figure 14, studies performed using the Whole PTH assay show that hPTH 1-84 was detected with a high degree of sensitivity, whereas hPTH 7-84 was undetectable, even at a concentration as high as 10,000 pg/mL.

1. STUDIES *IN VITRO*

[00155] The results of cAMP production by ROS/17.2 cells exposed to hPTH 1-84 or hPTH 7-84 are shown in Figure 15. Unlike hPTH 7-84, hPTH 1-84 increased cAMP production in a does-dependent manner. hPTH 1-84 (10^{-8} mol/L) increased intracellular cAMP from 18.1 ± 1.25 to 738 ± 4.13 nmol/well. On the other hand, the same concentration of hPTH 7-84 had no effect on cAMP ($N = 6$).

2. STUDIES *IN VIVO* IN RATS

[00156] We next examined the hPTH 7-84 fragment as a potential competitive inhibitor of hPTH 1-84 in bone by measuring changes in serum calcium in PTX rats. Figure 12 shows that the administration of hPTH 1-84 to PTX rats fed a 0.02% calcium diet increased plasma calcium by 0.65±0.10 mg/dL (N=9, P.< 0.001, ANOVA). With the administration of vehicle alone, plasma calcium changed slightly in accordance with PTX (-0.17 ± 0.10 mg/dL, $N = 5$). A slight but significant decrease was observed in the rats receiving hPTH 7-84 (0.30 ± 0.08 mg/dL, $N = 5$, $P < 0.05$). When both peptides were given together in a 1:1 molar ratio, the calcemic response induced by the administration of hPTH 1-84 alone decreased by 94% ($N = 6$, $P < 0.001$, ANOVA. Thus in this model, hPTH 7-84 significantly inhibits hPTH 1-84 induction of bone calcium mobilization.

[00157] The phosphaturic effects of these two peptides were then evaluated (Fig. 16). The GFR did not change in rats infused with hPTH 1-84 (1.8 ± 0.3 vs. 1.8 ± 0.1 mL/min), whereas fractional excretion of phosphate (FE_{P04}) increased from 11.9 ± 2.4 to 27.7 ± 2.4% ($N = 10$, $P < 0.001$). When hPTH 7-84 was given simultaneously with hPTH 1-84, the GFR increased from 2.1 ± 0.1 to 2.6 ± 0.2 mL/min ($N = 8$, $P < 0.05$). However, despite this increase

in GFR, the increase in FE_{PO4} induced by treatment with hPTH 1-84 was significantly decreased by 50.2% ($P < 0.01$). by virtue of the co-administration of hPTH 7-84.

3. STUDIES IN HUMANS

[00158] Figure 17 shows that the values for plasma PTH were higher in all 28 patients on chronic dialysis when measured with the I-Nichols assay compared with the Whole assay. The median PTH values were 523 versus 344 pg/ml ($P < 0.001$), respectively. A regression analysis of these data is shown in Figure 7.

[00159] The association between plasma levels of non-(1-84) PTH, "likely" hPTH 7-84, and plasma calcium and phosphorus was next examined in 20 patients maintained on chronic dialysis (Fig. 18). There was a positive correlation between the percentage of non- (1-84) PTH and serum calcium ($P < 0.002$), but no correlation with plasma phosphorus (data not shown). These studies were performed only in those patients in whom there were values for calcium, phosphorous, and PTH from the same blood sample [20].

[00160] In a group of 14 renal transplant patients the percentage of non-(1-84) PTH was found to be 44.1 ± 3.1% of the total PTH, as measured by the I-Nichols assay and the Whole PTH assay (Fig. 19). The absolute PTH value with the I-Nichols assay was 132.9 ± 39.9 compared with 79.8 ± 24.8 pg/mL ($P < 0.005$) with the Whole PTH assay.

[00161] Finally, we examined whether intracellular cleavage of the hPTH 1-84 molecule occurs in the parathyroid gland, thus producing the non (1-84) PTH fragment. Surgically excised parathyroid glands from six uremic patients maintained on chronic dialysis were studied. Figure 20 shows that non (1-84) PTH fragments exist in the cell lysates from these parathyroid glands and represent 41.8 ± 3.2% ($P < 0.05$) of the total intracellular PTH measured by the "intact" PTH assay (that is, 1-84 PTH and most likely 7-84 PTH).

[00162] The ordinarily skilled artisan can appreciate that the present invention can incorporate any number of the preferred features described above.

[00163] The above examples are included for illustrative purposes only and are not intended to limit the scope of the invention. Many variations to those described above are possible. Since modifications and variations to the examples described above will be apparent to those of skill in this art, it is intended that this invention be limited only by the scope of the appended claims.

[00164] Citation of the above publications or documents is not intended as an admission that any of the foregoing is pertinent prior art, nor does it constitute any admission as to the contents or date of these publications or documents.

What is claimed is:

1. An isolated antibody that specifically binds to an N-terminal sequence of whole parathyroid hormone (PTH) and is capable of detecting said whole PTH at a physiological level in a mammalian sample, with a proviso that said isolated antibody avoids binding to a non-whole PTH fragment.

2. The isolated antibody of claim 1, which is a monoclonal or polyclonal antibody or antibody fragment.

3. The isolated antibody of claim 1, which specifically binds to an epitope comprised in PTH_{1-6}, PTH_{1-8}, PTH_{1-9}, PTH_{1-12}, PTH_{1-15} or PTH_{3-12}.

4. The isolated antibody of claim 1, which specifically binds to the parathyroid hormone peptide human PTH_{1-8}, rat PTH_{1-8}, mouse PTH_{1-8}, bovine PTH_{1-8}, canine PTH_{1-8}, porcine PTH_{1-8}, horse PTH_{1-8}, human PTH_{1-15}, rat PTH_{1-15}, mouse PTH_{1-15}, bovine PTH_{1-15}, canine PTH_{1-15}, porcine PTH_{1-15}, or horse PTH_{1-15}, wherein at least four amino acids in said peptide sequence are part of a reactive portion with the antibody.

5. The isolated antibody of claim 1, which specifically binds to an epitope comprised in PTH_{1-5}, PTH_{1-7}, PTH_{1-8}, PTH_{1-10}, PTH_{1-11}, PTH_{1-13}, PTH_{1-14}, PTH_{1-15}, PTH_{1-16}, PTH_{1-17}, PTH_{1-18}, PTH_{1-19}, PTH_{1-20}, PTH_{1-21}, PTH_{1-22}, PTH_{1-23}, PTH_{1-24}, PTH_{1-25}, PTH_{1-26}, $hPTH_{1-27}$, PTH_{1-28}, PTH_{1-29}, PTH_{1-30}, PTH_{1-31}, PTH_{1-32}, PTH_{1-33}, PTH_{1-34}, PTH_{1-35}, PTH_{1-36}, PTH_{1-37}, PTH_{2-5}, PTH_{2-6}, PTH_{2-7}, PTH_{2-8}, PTH_{2-9}, PTH_{2-10}, PTH_{2-11}, PTH_{2-12}, PTH_{2-13}, PTH_{2-14}, PTH_{2-15}, PTH_{2-16}, PTH_{2-17}, PTH_{2-18}, PTH_{2-19}, PTH_{2-20}, PTH_{2-21}, PTH_{2-22}, PTH_{2-23}, PTH_{2-24}, PTH_{2-25}, PTH_{2-26}, PTH_{2-27}, PTH_{2-28}, PTH_{2-29}, PTH_{2-30}, PTH_{2-31}, PTH_{2-32}, PTH_{2-33}, PTH_{2-34}, PTH_{2-35}, PTH_{2-36}, PTH_{2-37}, PTH_{3-6}, PTH_{3-7}, PTH_{3-8}, PTH_{3-9}, PTH_{3-10}, PTH_{3-11}, PTH_{3-13}, PTH_{3-14}, PTH_{3-15}, PTH_{3-16}, PTH_{3-17}, PTH_{3-18}, PTH_{3-19}, PTH_{3-20}, PTH_{3-21}, PTH_{3-22}, PTH_{3-23}, PTH_{3-24}, PTH_{3-25}, PTH_{3-26}, PTH_{3-27}, PTH_{3-28}, PTH_{3-29}, PTH_{3-30}, PTH_{3-31}, PTH_{3-32}, PTH_{3-33}, PTH_{3-34}, PTH_{3-35}, PTH_{3-36}, PTH_{3-37}, PTH_{4-7}, PTH_{4-8}, PTH_{4-9}, PTH_{4-10}, PTH_{4-11}, PTH_{4-12}, PTH_{4-13}, PTH_{4-14}, PTH_{4-15}, PTH_{4-16}, PTH_{4-17}, PTH_{4-18}, PTH_{4-19}, PTH_{4-20}, PTH_{4-21}, PTH_{4-22}, PTH_{4-23}, PTH_{4-24}, PTH_{4-25}, PTH_{4-26}, PTH_{4-27}, PTH_{4-28}, PTH_{4-29}, PTH_{4-30}, PTH_{4-31}, PTH_{4-32}, PTH_{4-33}, PTH_{4-34}, PTH_{4-35}, PTH_{4-36}, PTH_{4-37}, PTH_{5-8}, PTH_{5-9}, PTH_{5-10}, PTH_{5-11}, PTH_{5-12}, PTH_{5-13}, PTH_{5-14}, PTH_{5-15}, PTH_{5-16}, PTH_{5-17}, PTH_{5-18}, PTH_{5-19}, PTH_{5-20}, PTH_{5-21}, PTH_{5-22}, PTH_{5-23}, PTH_{5-24}, PTH_{5-25}, PTH_{5-26}, PTH_{5-27}, PTH_{5-28}, PTH_{5-29}, PTH_{5-30}, PTH_{5-31}, PTH_{5-32}, PTH_{5-33}, PTH_{5-34}, PTH_{5-35}, PTH_{5-36}, or PTH_{5-37}.

6. The isolated antibody of claim 1, wherein the binding between the antibody and the N-terminal sequence of whole PTH is dependent on the presence of amino acid residues 2-5 of the hPTH.

7. The isolated antibody of claim 1, wherein the binding between the antibody and the N-terminal sequence of whole PTH is dependent on the presence of amino acid residues 3-6 of the hPTH.

8. The isolated antibody or antibody fragment of claim 1, wherein the non-whole PTH fragment is a peptide having an amino acid sequence from between PTH_{3-84} and PTH_{34-84}.

9. The isolated antibody of claim 1, wherein the non-whole PTH fragment is a peptide having an amino acid sequence of human PTH_{7-84}.

10. A method for measuring a physiological level of whole parathyroid hormone in a mammalian sample, which method comprises:

a) obtaining a sample from a mammal to be tested;

b) contacting said sample with an isolated antibody that specifically binds to an N-terminal sequence of whole PTH and is capable of detecting said whole PTH at a physiological level in said mammalian sample, with a proviso that said isolated antibody avoids binding to a non-whole PTH fragment; and

c) assessing a complex formed between said whole parathyroid hormone, if present in said sample, and said antibody, to measure physiological level of said whole parathyroid hormone in said mammalian sample.

11. The method of claim 10, wherein the sample is selected from the group consisting of a serum, a plasma and a blood sample.

12. The method of claim 10, wherein the sample is a clinical sample.

13. The method of claim 10 which is used for clinical management of renal disease subjects, subjects afflicted with osteoporosis or diagnosing primary hyperparathyroidism.

14. The method of claim 10, wherein the mammal is a human.

15. The method of claim 14, wherein the sample is a human clinical sample.

16. The method of claim 10, wherein the antibody is a monoclonal or polyclonal antibody or antibody fragment.

17. The method of claim 10, wherein the antibody specifically binds to an epitope comprised in PTH_{1-6}, PTH_{1-8}, PTH_{1-9}, PTH_{1-12}, PTH_{1-15}, or PTH_{3-12}.

18. The method of claim 10, wherein the antibody specifically binds to the parathyroid hormone peptide human PTH_{1-8}, rat PTH_{1-8}, mouse PTH_{1-8}, bovine PTH_{1-8}, canine PTH_{1-8}, porcine PTH_{1-8}, horse PTH_{1-8}, human PTH_{1-15}, rat PTH_{1-15}, mouse PTH_{1-15}, bovine PTH_{1-15}, canine PTH_{1-15}, porcine PTH_{1-15}, or horse PTH_{1-15}, wherein at least four amino acids in said peptide sequence are part of a reactive portion with the antibody.

19. The method of claim 10, wherein the antibody specifically binds to an epitope comprised in PTH_{1-5}, PTH_{1-7}, PTH_{1-8}, PTH_{1-10}, PTH_{1-11}, PTH_{1-13}, PTH_{1-14}, PTH_{1-15}, PTH_{1-16}, PTH_{1-17}, PTH_{1-18}, PTH_{1-19}, PTH_{1-20}, PTH_{1-21}, PTH_{1-22}, PTH_{1-23}, PTH_{1-24}, PTH_{1-25}, PTH_{1-26}, $hPTH_{1-27}$, PTH_{1-28}, PTH_{1-29}, PTH_{1-30}, PTH_{1-31}, PTH_{1-32}, PTH_{1-33}, PTH_{1-34}, PTH_{1-35}, PTH_{1-36}, PTH_{1-37}, PTH_{2-5}, PTH_{2-6}, PTH_{2-7}, PTH_{2-8}, PTH_{2-9}, PTH_{2-10}, PTH_{2-11}, PTH_{2-12}, PTH_{2-13}, PTH_{2-14}, PTH_{2-15}, PTH_{2-16}, PTH_{2-17}, PTH_{2-18}, PTH_{2-19}, PTH_{2-20}, PTH_{2-21}, PTH_{2-22}, PTH_{2-23}, PTH_{2-24}, PTH_{2-25}, PTH_{2-26}, PTH_{2-27}, PTH_{2-28}, PTH_{2-29}, PTH_{2-30}, PTH_{2-31}, PTH_{2-32}, PTH_{2-33}, PTH_{2-34}, PTH_{2-35}, PTH_{2-36}, PTH_{2-37}, PTH_{3-6}, PTH_{3-7}, PTH_{3-8}, PTH_{3-9}, PTH_{3-10}, PTH_{3-11}, PTH_{3-13}, PTH_{3-14}, PTH_{3-15}, PTH_{3-16}, PTH_{3-17}, PTH_{3-18}, PTH_{3-19}, PTH_{3-20}, PTH_{3-21}, PTH_{3-22}, PTH_{3-23}, PTH_{3-24}, PTH_{3-25}, PTH_{3-26}, PTH_{3-27}, PTH_{3-28}, PTH_{3-29}, PTH_{3-30}, PTH_{3-31}, PTH_{3-32}, PTH_{3-33}, PTH_{3-34}, PTH_{3-35}, PTH_{3-36}, PTH_{3-37}, PTH_{4-7}, PTH_{4-8}, PTH_{4-9}, PTH_{4-10}, PTH_{4-11}, PTH_{4-12}, PTH_{4-13}, PTH_{4-14}, PTH_{4-15}, PTH_{4-16}, PTH_{4-17}, PTH_{4-18}, PTH_{4-19}, PTH_{4-20}, PTH_{4-21}, PTH_{4-22}, PTH_{4-23}, PTH_{4-24}, PTH_{4-25}, PTH_{4-26}, PTH_{4-27}, PTH_{4-28}, PTH_{4-29}, PTH_{4-30}, PTH_{4-31}, PTH_{4-32}, PTH_{4-33}, PTH_{4-34}, PTH_{4-35}, PTH_{4-36}, PTH_{4-37}, PTH_{5-8}, PTH_{5-9}, PTH_{5-10}, PTH_{5-11}, PTH_{5-12}, PTH_{5-13}, PTH_{5-14}, PTH_{5-15}, PTH_{5-16}, PTH_{5-17}, PTH_{5-18}, PTH_{5-19}, PTH_{5-20}, PTH_{5-21}, PTH_{5-22}, PTH_{5-23}, PTH_{5-24}, PTH_{5-25}, PTH_{5-26}, PTH_{5-27}, PTH_{5-28}, PTH_{5-29}, PTH_{5-30}, PTH_{5-31}, PTH_{5-32}, PTH_{5-33}, PTH_{5-34}, PTH_{5-35}, PTH_{5-36}, or PTH_{5-37}.

20. The method of claim 19, wherein the binding between the antibody and the N-terminal sequence of whole PTH is dependent on the presence of amino acid residues 2-5 of the hPTH.

21. The method of claim 19, wherein the binding between the antibody and the N-terminal sequence of whole PTH is dependent on the presence of amino acid residues 3-6 of the hPTH.

22. The method of claim 10, wherein the non-whole PTH fragment is a peptide having an amino acid sequence from between PTH_{3-84} and PTH_{34-84}.

23. The method of claim 10, wherein the non-whole PTH fragment is a peptide having an amino acid sequence of human PTH_{7-84}.

24. The method of claim 10, wherein the complex is assessed by a sandwich or competitive assay format.

25. The method of claim 24, wherein the antibody that specifically binds to an N-terminal sequence of whole PTH is used as a first antibody and an antibody that is capable of binding to a portion of whole PTH other than the N-terminal sequence which binds to the first antibody is used as a second antibody in a sandwich assay format.

26. The method of claim 25, wherein either the first antibody or the second antibody is attached to a surface and functions as a capture antibody.

27. The method of claim 26, wherein the capture antibody is attached to the surface directly or indirectly.

28. The method of claim 26, wherein the capture antibody is attached to the surface via a biotin-avidin (or streptavidin) linking pair.

29. The method of claim 10, wherein the complex is assessed by a format selected from the group consisting of an enzyme-linked immunosorbent assay (ELISA), immunoblotting, immunoprecipitation, radioimmunoassay (RIA), immunostaining, latex agglutination, indirect hemagglutination assay (IHA), complement fixation, indirect immunofluorescent assay (IFA), nephelometry, flow cytometry assay, plasmon resonance assay, chemiluminescence assay, lateral flow immunoassay, u-capture assay, inhibition assay and avidity assay.

30. The method of claim 10, wherein the complex is assessed in a homogeneous or a heterogeneous assay format.

31. The method of claim 10, wherein the physiological level of whole parathyroid hormone is less than 4 pmol/L.

32. The method of claim 10, wherein the physiological level of whole parathyroid hormone is from about 0.2 pmol/L to about 4 pmol/L.

33. The method of claim 10, which further comprises measuring a PTH peptide fragment level and/or total PTH level.

34. The method of claim 33, wherein said sample is contacted with one or more isolated antibodies, and wherein each of said one or more isolated antibodies specifically bind

one or more PTH peptide fragments selected from the group consisting of: PTH_{39-84}, PTH_{1-34}, PTH_{43-68}, PTH_{7-84}, PTH_{39-68}, PTH_{53-84}, PTH_{65-84}, PTH_{44-68}, PTH_{19-84}, PTH_{23-84}, PTH_{1-38}, PTH_{1-48}, PTH_{1-58}, PTH_{1-68}, and PTH_{1-78}.

35. The method of claim 33, which further comprises comparing at least two parameters selected from the group consisting of the whole PTH level, total PTH peptide fragment level, total PTH level, C-terminal PTH fragment (cPTH) level, N-terminal PTH fragment level, and mid-terminal PTH fragment (mPTH) level.

36. The method of claim 35, wherein the results of said comparison are used to determine whether the mammal suffers from a bone turnover related disorder, or to monitor bone disease or disorder related treatment.

37. The method of claim 36, which is used in the diagnosis or monitoring of treatment for adynamic bone disease (ADN) or severe hyperparathyroidism.

38. The method of claim 35, wherein the comparison is in the form of a ratio or proportion between the whole PTH level and the total PTH level.

39. The method of claim 35, wherein the comparison is in the form of a ratio or proportion between the whole PTH level versus the combined total of the total PTH level minus the whole PTH level.

40. The method of claim 35, wherein the comparison is in the form of a ratio or proportion between the wPTH level versus the combined cPTH and mPTH fragment levels.

41. The method of claim 40, wherein the comparison is a ratio having a value less than about 0.020, and wherein the mammal is determined to be afflicted with adynamic bone disease.

42. The method of claim 40, wherein the comparison is a ratio having a value greater than about 0.020, and wherein the mammal is determined to be afflicted with severe hyperparathyroidism.

43. The method of claim 35, wherein the comparison is in the form of a ratio or proportion represented by the equation: wPTH / ((cPTH-wPTH)+(mPTH-wPTH)).

44. The method of claim 43, wherein the comparison is a ratio having a value less than about 0.0185, and wherein the mammal is determined to be afflicted with adynamic bone disease.

45. The method of claim 43, wherein the comparison is a ratio having a value greater than about 0.0185, and wherein the mammal is determined to be afflicted with severe hyperparathyroidism.

46. The method of claim 35, wherein the comparison is in the form of a ratio or proportion between the whole PTH level versus the total of the combined cPTH and mPTH fragment levels subtracted by the whole PTH level.

47. The method of claim 46, wherein the comparison is a ratio having a value less than about 0.020, and wherein the mammal is determined to be afflicted with adynamic bone disease.

48. The method of claim 46, wherein the comparison is a ratio having a value greater than about 0.020, and wherein the mammal is determined to be afflicted with severe hyperparathyroidism.

49. The method of claim 35, wherein the comparison is in the form of a ratio or proportion between the whole PTH level versus the combined whole PTH level, cPTH and mPTH fragment levels.

50. The method of claim 49, wherein the comparison is a ratio having a value less than about 0.0175, and wherein the mammal is determined to be afflicted with adynamic bone disease.

51. The method of claim 49, wherein the comparison is a ratio having a value greater than about 0.0175, and wherein the mammal is determined to be afflicted with severe hyperparathyroidism.

52. The method of claim 35, wherein the comparison is in the form of a ratio or proportion between the whole PTH level versus the cPTH fragment level.

53. The method of claim 52, wherein the comparison is a ratio having a value less than about 0.103, and wherein the mammal is determined to be afflicted with adynamic bone disease.

54. The method of claim 52, wherein the comparison is a ratio having a value greater than about 0.103, and wherein the mammal is determined to be afflicted with severe hyperparathyroidism.

55. The method of claim 35, wherein the comparison is in the form of a ratio or proportion between the whole PTH level versus the mPTH fragment level.

56. The method of claim 55, wherein the comparison is a ratio having a value less than about 0.0225, and wherein the mammal is determined to be afflicted with adynamic bone disease.

57. The method of claim 55, wherein the comparison is a ratio having a value greater than about 0.0225, and wherein the mammal is determined to be afflicted with severe hyperparathyroidism.

58. The method of claim 10, which is used for:

a) differentiating between a person having substantially normal parathyroid function and having hyperparathyroidism;

b) monitoring parathyroid related bone disease and treatment;

c) monitoring effects of therapeutic treatment for hyperparathyroidism; or

d) diagnosing parathyroid related bone disease.

59. A kit for measuring a physiological level of whole parathyroid hormone in a mammalian sample, which kit comprises, in a container, an isolated antibody that specifically binds to an N-terminal sequence of whole parathyroid hormone (PTH) and is capable of detecting said whole PTH at a physiological level in a mammalian sample, with a proviso that said isolated antibody avoids binding to a non-whole PTH fragment.

60. An isolated parathyroid hormone (PTH) peptide, which is selected from the group consisting of PTH_{1-11}, PTH_{1-13}, PTH_{1-14}, PTH_{1-15}, PTH_{1-16}, PTH_{1-17}, PTH_{1-18}, PTH_{1-19}, PTH_{1-20}, PTH_{1-21}, PTH_{1-22}, PTH_{1-23}, PTH_{1-24}, PTH_{1-25}, PTH_{1-26}, $hPTH_{1-27}$, PTH_{1-28}, PTH_{1-29}, PTH_{1-30}, PTH_{1-31}, PTH_{1-32}, PTH_{1-33}, PTH_{1-34}, PTH_{1-35}, PTH_{1-36}, PTH_{2-5}, PTH_{2-6}, PTH_{2-8}, PTH_{2-9}, PTH_{2-10}, PTH_{2-11}, PTH_{2-12}, PTH_{2-13}, PTH_{2-14}, PTH_{2-15}, PTH_{2-16}, PTH_{2-17}, PTH_{2-18}, PTH_{2-19}, PTH_{2-20}, PTH_{2-21}, PTH_{2-22}, PTH_{2-23}, PTH_{2-24}, PTH_{2-25}, PTH_{2-26}, PTH_{2-27}, PTH_{2-28}, PTH_{2-29}, PTH_{2-30}, PTH_{2-31}, PTH_{2-32}, PTH_{2-33}, PTH_{2-34}, PTH_{2-35}, PTH_{2-36}, PTH_{3-6}, PTH_{3-7}, PTH_{3-9}, PTH_{3-10}, PTH_{3-11}, PTH_{3-12}, PTH_{3-13}, PTH_{3-14}, PTH_{3-15}, PTH_{3-16}, PTH_{3-17}, PTH_{3-18}, PTH_{3-19}, PTH_{3-20}, PTH_{3-21}, PTH_{3-22}, PTH_{3-23}, PTH_{3-24}, PTH_{3-25}, PTH_{3-26}, PTH_{3-27}, PTH_{3-28}, PTH_{3-29}, PTH_{3-30}, PTH_{3-31}, PTH_{3-32}, PTH_{3-33}, PTH_{3-34}, PTH_{3-35}, PTH_{3-36}, PTH_{4-7}, PTH_{4-8}, PTH_{4-9}, PTH_{4-10}, PTH_{4-11}, PTH_{4-13}, PTH_{4-14},

PTH_{4-15}, PTH_{4-16}, PTH_{4-17}, PTH_{4-18}, PTH_{4-19}, PTH_{4-20}, PTH_{4-21}, PTH_{4-22}, PTH_{4-23}, PTH_{4-24}, PTH_{4-25}, PTH_{4-26}, PTH_{4-27}, PTH_{4-28}, PTH_{4-29}, PTH_{4-30}, PTH_{4-31}, PTH_{4-32}, PTH_{4-33}, PTH_{4-34}, PTH_{4-35}, PTH_{4-36}, PTH_{5-8}, PTH_{5-9}, PTH_{5-11}, PTH_{5-12}, PTH_{5-13}, PTH_{5-14}, PTH_{5-15}, PTH_{5-16}, PTH_{5-17}, PTH_{5-18}, PTH_{5-19}, PTH_{5-20}, PTH_{5-21}, PTH_{5-22}, PTH_{5-23}, PTH_{5-24}, PTH_{5-25}, PTH_{5-26}, PTH_{5-27}, PTH_{5-28}, PTH_{5-29}, PTH_{5-30}, PTH_{5-31}, PTH_{5-32}, PTH_{5-33}, PTH_{5-34}, PTH_{5-35}, PTH_{5-36}, and PTH_{5-37}.

61. The isolated PTH peptide of claim 60, which is conjugated to a carrier to enhance the PTH peptide's immunogenecity.

62. The isolated PTH peptide of claim 61, wherein the carrier is a carrier protein.

63. The isolated PTH peptide of claim 62, wherein the PTH peptide and the carrier protein are parts of a fusion protein.

64. An immunogen, which immunogen comprises:

a) a PTH peptide of claim 60; and

b) an immune response potentiator.

65. The immunogen of claim 64, wherein the immune response potentiator is selected from the group consisting of Bacille Calmette-Guerin (BCG), Corynebacterium Parvum, Brucella abortus extract, glucan, levamisole, tilorone, an enzyme and a non-virulent virus.

66. A multiple antigenic peptide (MAP), which MAP comprises a branched oligolysine core conjugated with a plurality of the PTH peptide of claim 60.

67. The MAP of claim 66, wherein the branched oligolysine core comprises 3, 7 or 15 lysine residues.

68. The MAP of claim 66, wherein the plurality of the PTH peptide is conjugated to the branched oligolysine core via a spacer.

69. The MAP of claim 68, wherein the spacer is an amino acid residue.

70. The MAP of claim 68, which comprises 4, 8 or 16 copies of the PTH peptide.

71. The MAP of claim 66, wherein the plurality of the PTH peptide comprises same or different PTH peptides.

72. A method for producing an antibody to a parathyroid hormone (PTH) or a PTH peptide, which method comprises:

a) introducing an isolated PTH peptide of claim 60 to a mammal in an amount sufficient to produce an antibody to said PTH peptide; and

b) recovering said antibody from said mammal.

73. An antibody to a PTH or a PTH peptide produced by the method of claim 72.

74. A kit for producing an antibody to a parathyroid hormone (PTH) or a PTH peptide, which kit comprises:

a) an isolated PTH peptide of claim 60;

b) means for introducing said isolated PTH peptide to a mammal in an amount sufficient to produce an antibody to said PTH peptide; and

c) means for recovering said antibody from said mammal.

75. A method for producing an antibody to a parathyroid hormone (PTH) or a PTH peptide, which method comprises:

a) introducing a MAP of claim 66 to a mammal in an amount sufficient to produce an antibody to a PTH peptide comprised in said MAP; and

b) recovering said antibody from said mammal.

76. An antibody to a PTH or a PTH peptide produced by the method of claim 75.

77. A kit for producing an antibody to a parathyroid hormone (PTH) or a PTH peptide, which kit comprises:

a) a MAP of claim 66;

b) means for introducing said MAP to a mammal in an amount sufficient to produce an antibody to a PTH peptide comprised in said MAP; and

c) means for recovering said antibody from said mammal.

78. A method for producing an antibody to a parathyroid hormone (PTH) or a PTH peptide, which method comprises:

a) introducing a PTH protein or peptide from between PTH_{1-34} and PTH_{1-84} to a mammal in an amount sufficient to produce an antibody to said PTH protein or peptide;

b) recovering said antibody from said mammal; and

c) affinity purifying a PTH antibody that specifically binds to an epitope comprised in a PTH peptide of claim 60 using said PTH peptide.

79. An antibody to a PTH or a PTH peptide produced by the method of claim 78.

80. A kit for producing an antibody to a parathyroid hormone (PTH) or a PTH peptide, which kit comprises:

a) a PTH protein or peptide from between PTH_{1-34} and PTH_{1-84};

b) means for introducing said PTH protein or peptide from between PTH_{1-34} and PTH_{1-84} to a mammal in an amount sufficient to produce an antibody to said PTH protein or peptide;

c) means for recovering said antibody from said mammal; and

d) a PTH peptide of claim 60.

81. The method of claim 10, wherein the physiological level of whole parathyroid hormone is from about 7 pgm/ml to about 39 pgm/ml.

FIG. 1

Whole Human PTH (1-84)

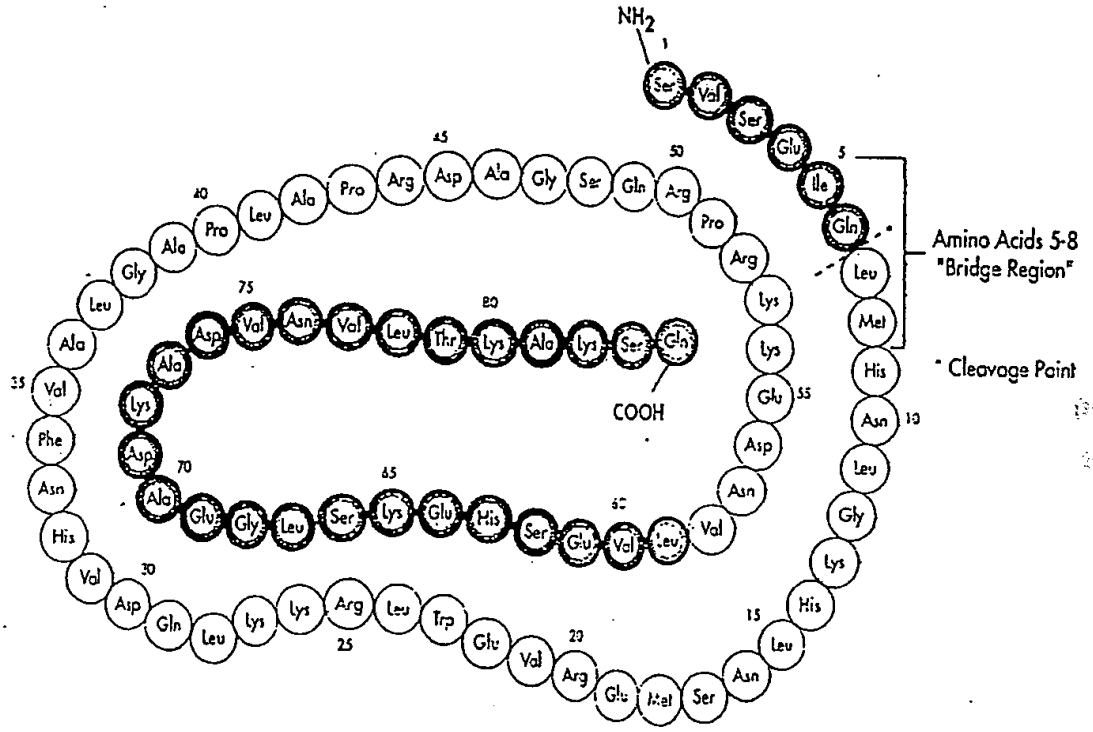


FIG. 2

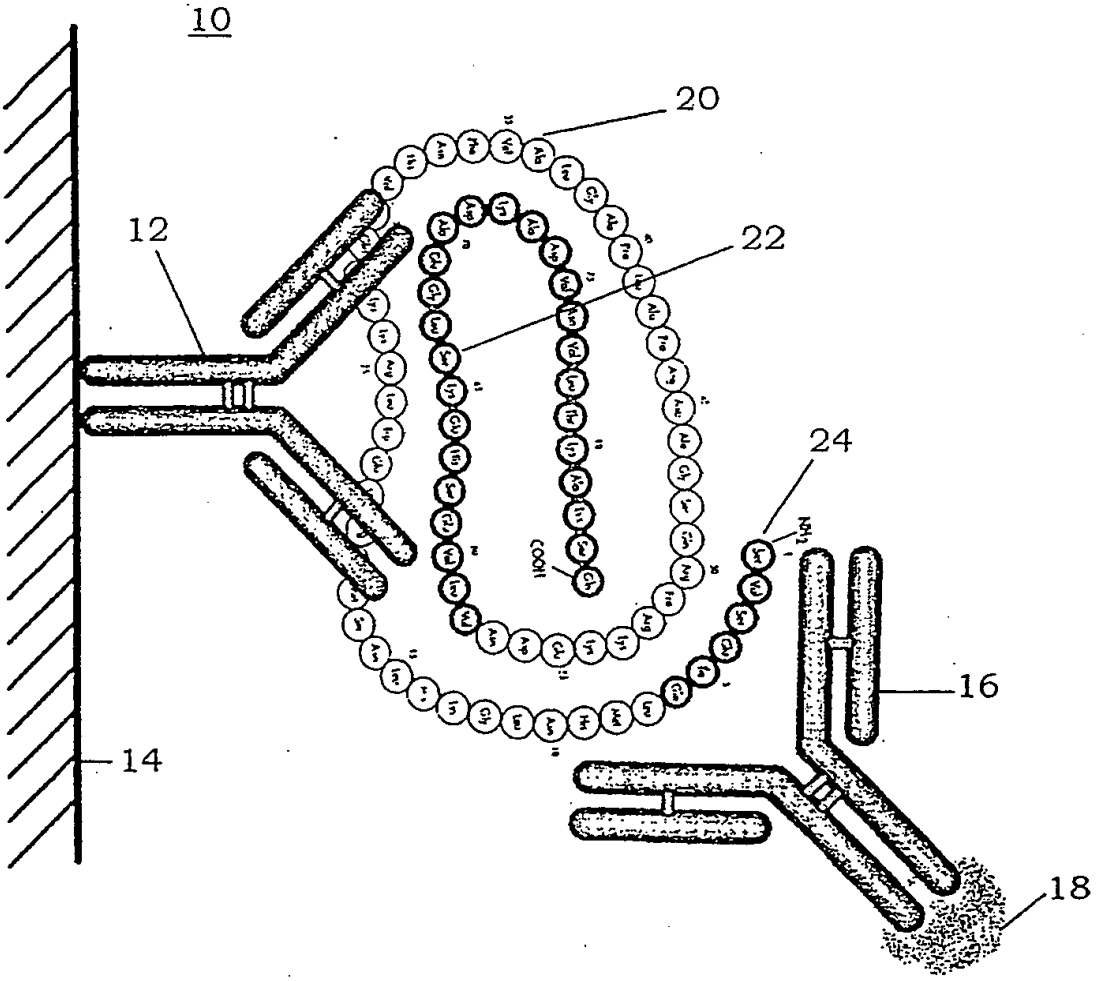


FIG. 3

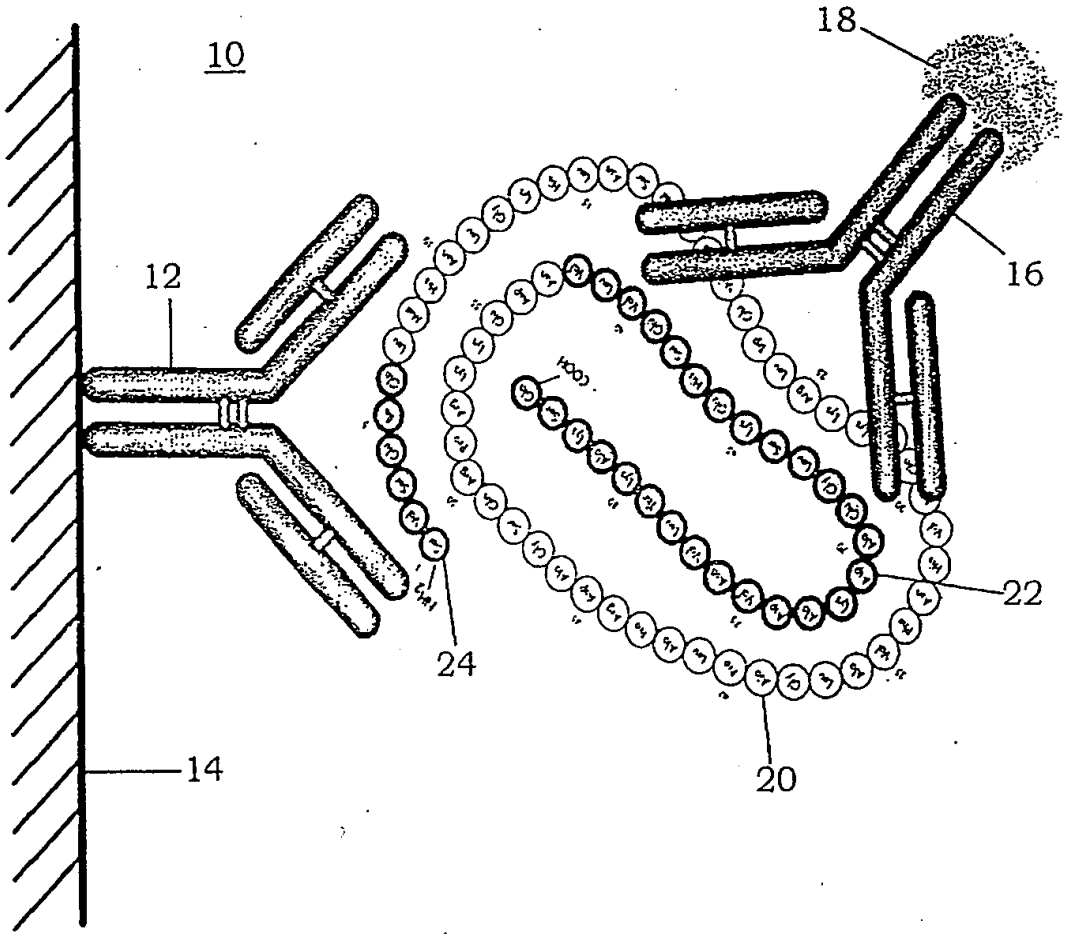


FIG. 4

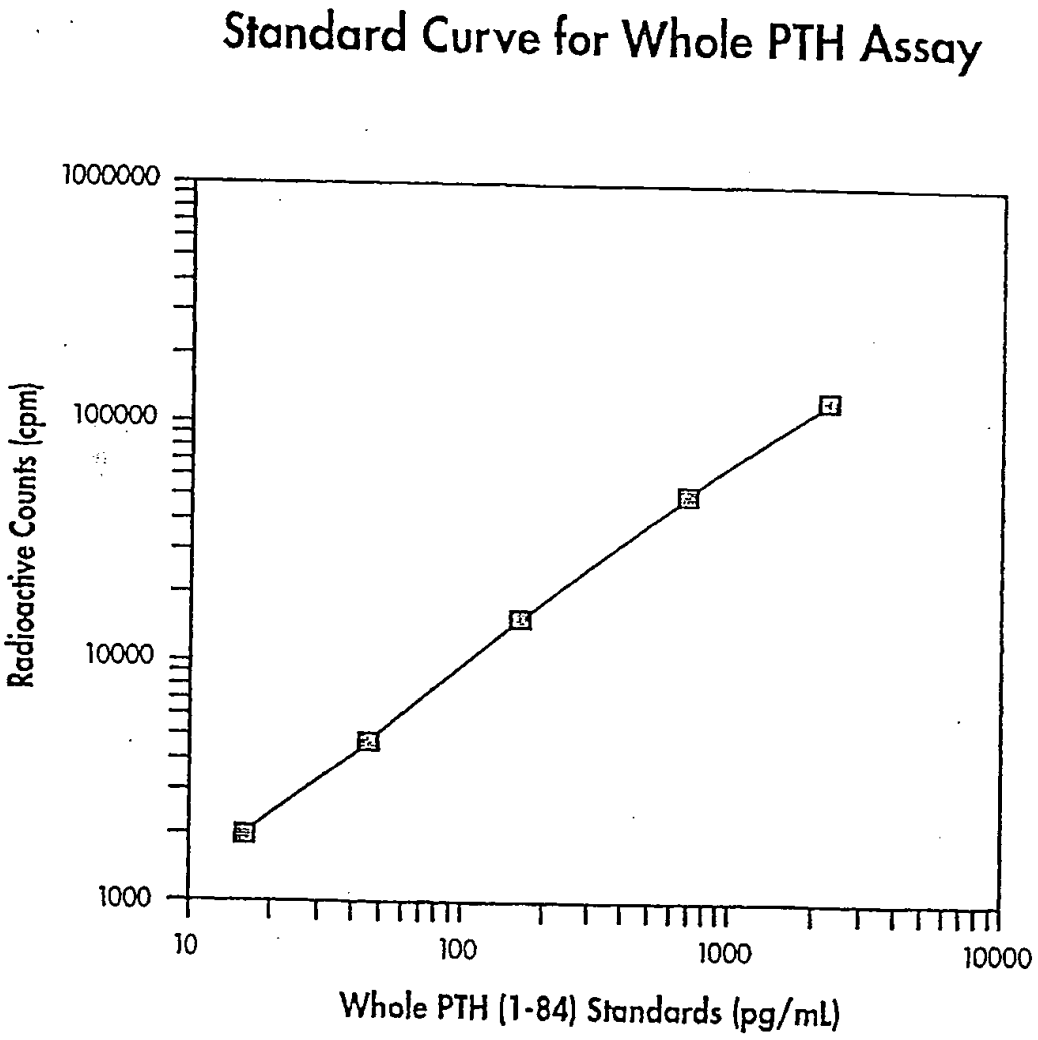

Standard Curve for Whole PTH Assay
1000000
100000
10000
1000
Radioactive Counts (cpm)
10
100
1000
10000
Whole PTH (1-84) Standards (pg/mL)

FIG. 5

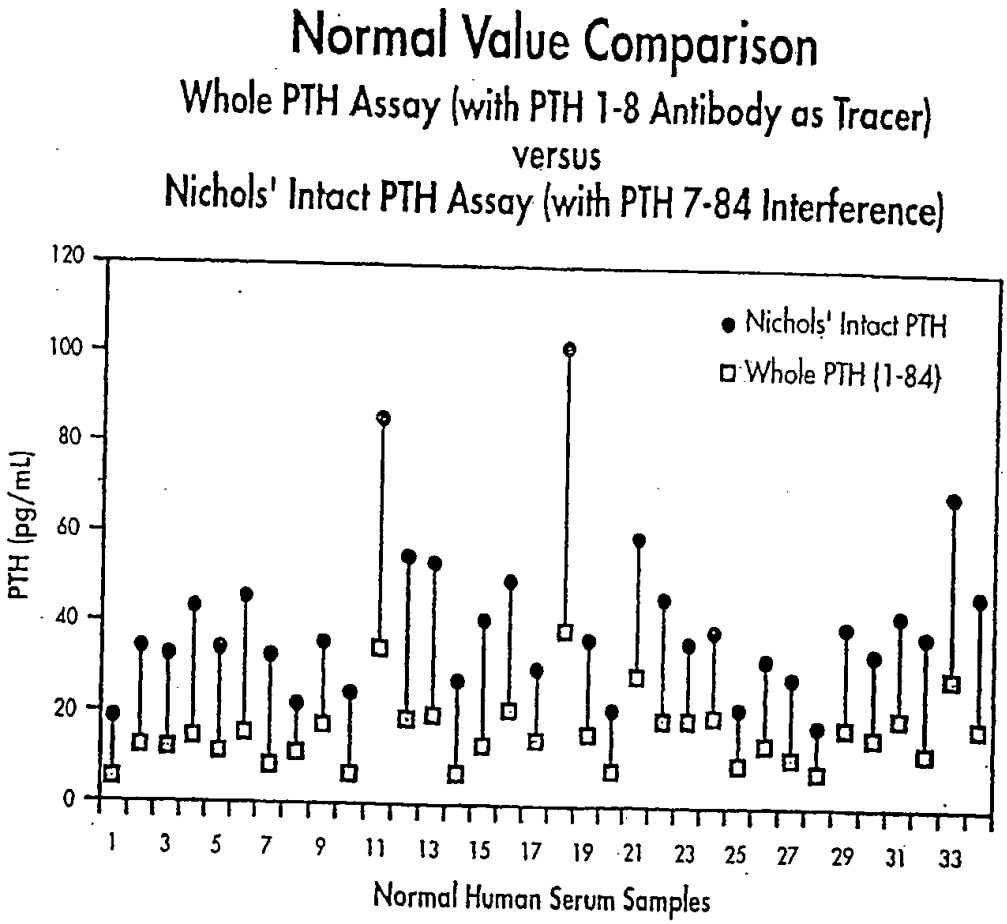

Normal Value Comparison
Whole PTH Assay (with PTH 1-8 Antibody as Tracer)
versus
Nichols' Intact PTH Assay (with PTH 7-84 Interference)
120
100
80
60
40
20
0
PTH (pg/mL)
● Nichols' Intact PTH
□ Whole PTH (1-84)
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33
Normal Human Serum Samples

FIG. 6A

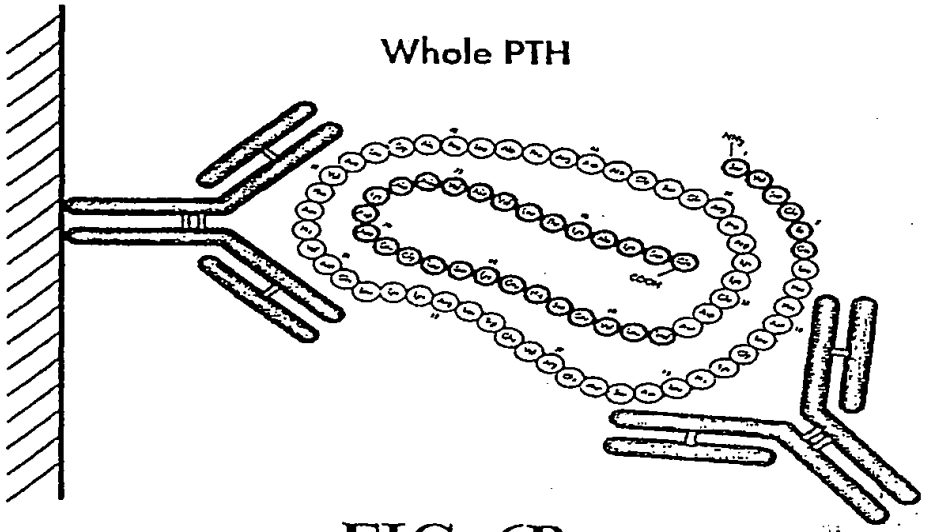


FIG. 6B

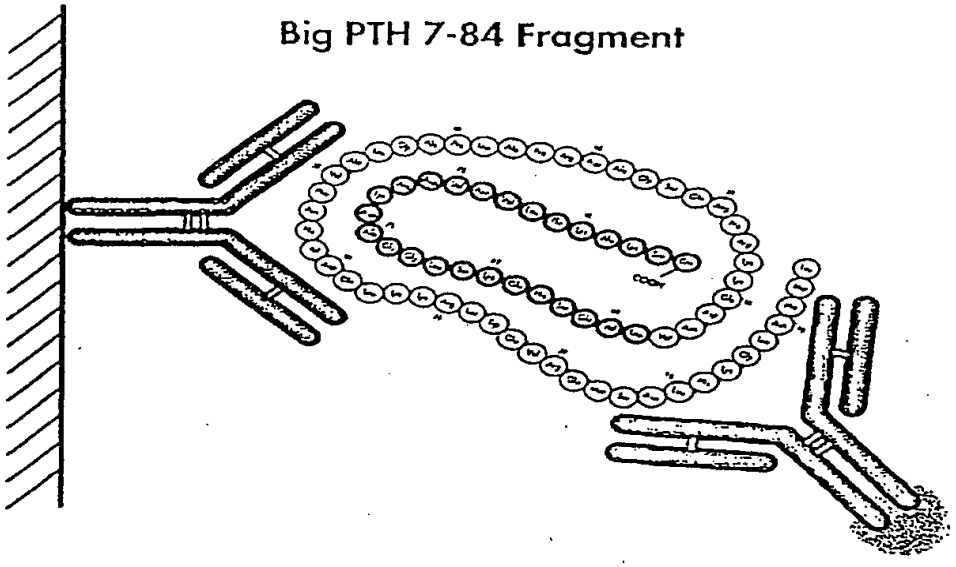


FIG. 7

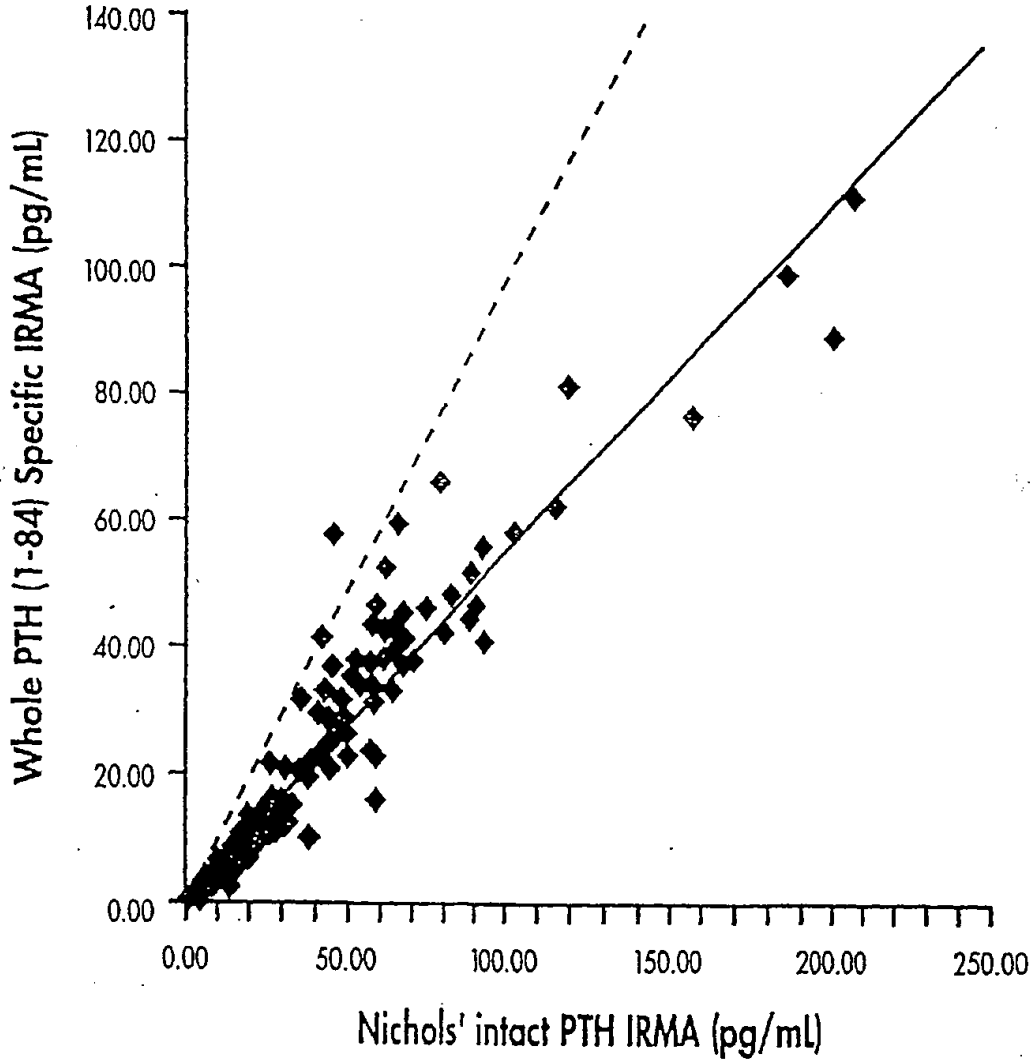

140.00
120.00
100.00
80.00
60.00
40.00
20.00
0.00
Whole PTH (1-84) Specific IRMA (pg/mL)
0.00
50.00
100.00
150.00
200.00
250.00
Nichols' intact PTH IRMA (pg/mL)

FIG. 8

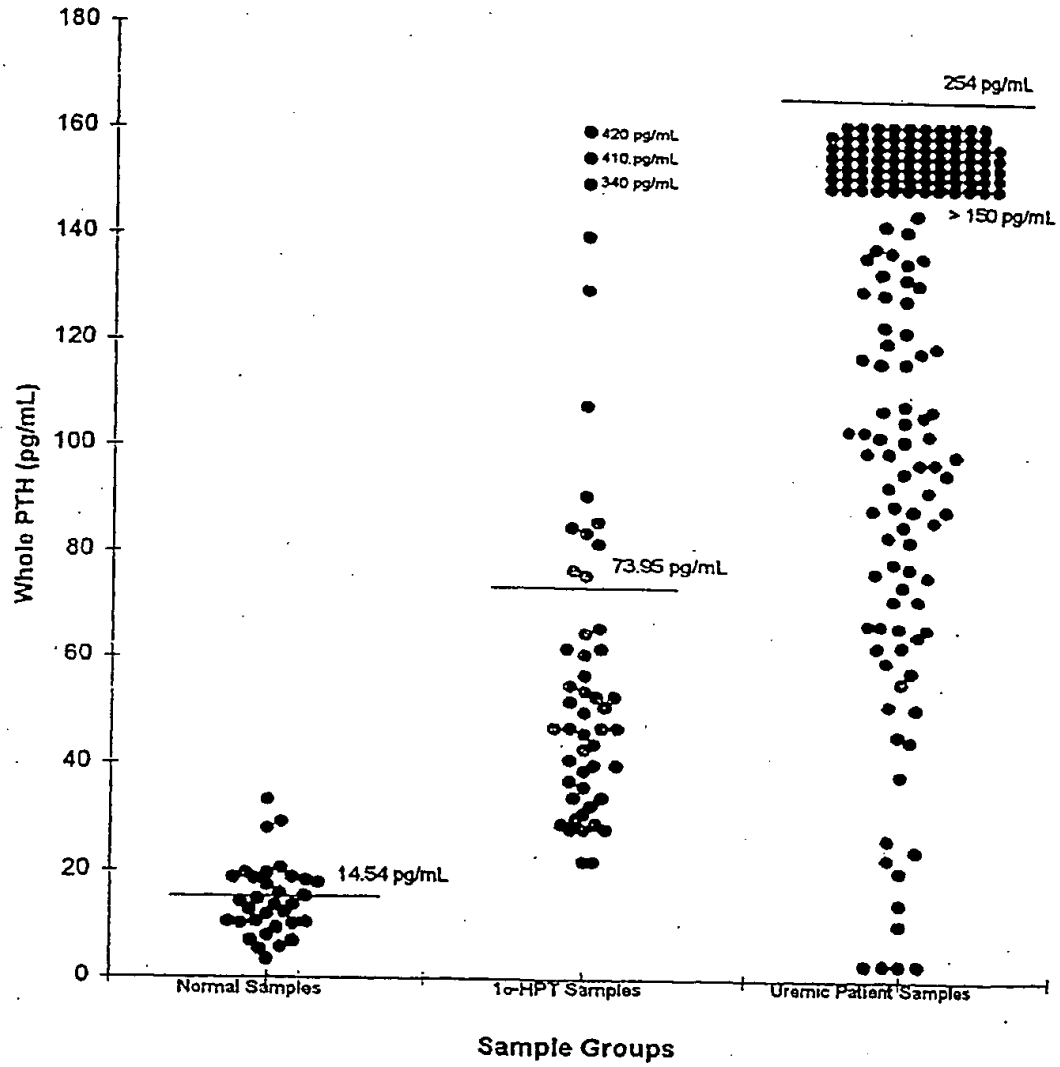

180
160
140
120
100
80
60
40
20
0
Whole PTH (pg/mL)
254 pg/mL
420 pg/mL
410 pg/mL
340 pg/mL
> 150 pg/mL
73.95 pg/mL
14.54 pg/mL
Normal Samples
1o-HPT Samples
Uremic Patient Samples
Sample Groups

FIG. 9

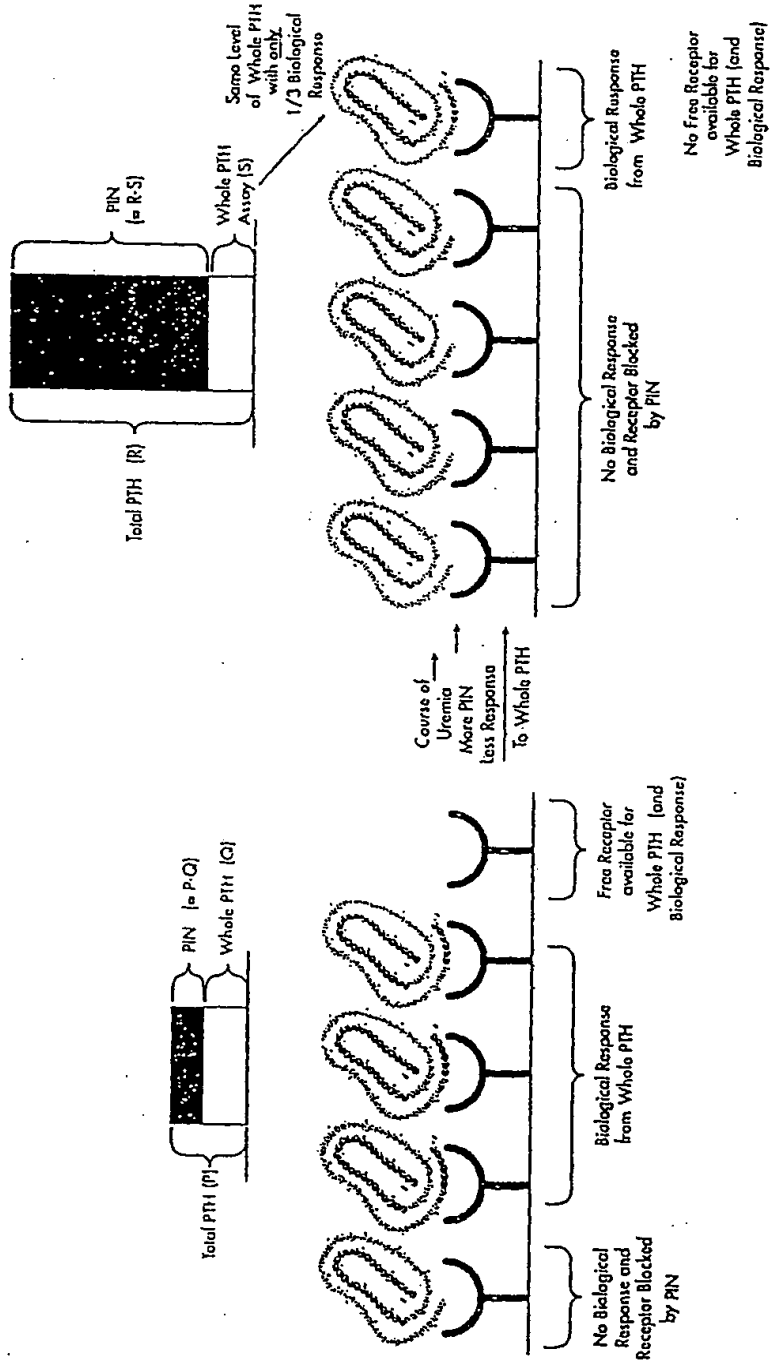

PIN (= R-S)
Whole PTH Assay (S)
Same Level of Whole PTH with only 1/3 Biological Response
Total PTH (R)
No Biological Response and Receptor Blocked by PIN
Biological Response from Whole PTH
Free Receptor available for Whole PTH (and Biological Response)
No Free Receptor available for Whole PTH (and Biological Response)
Course of Uremia
More PIN
Less Response
To Whole PTH
PIN (= P-Q)
Whole PTH (Q)
Total PTH (P)

FIG. 10

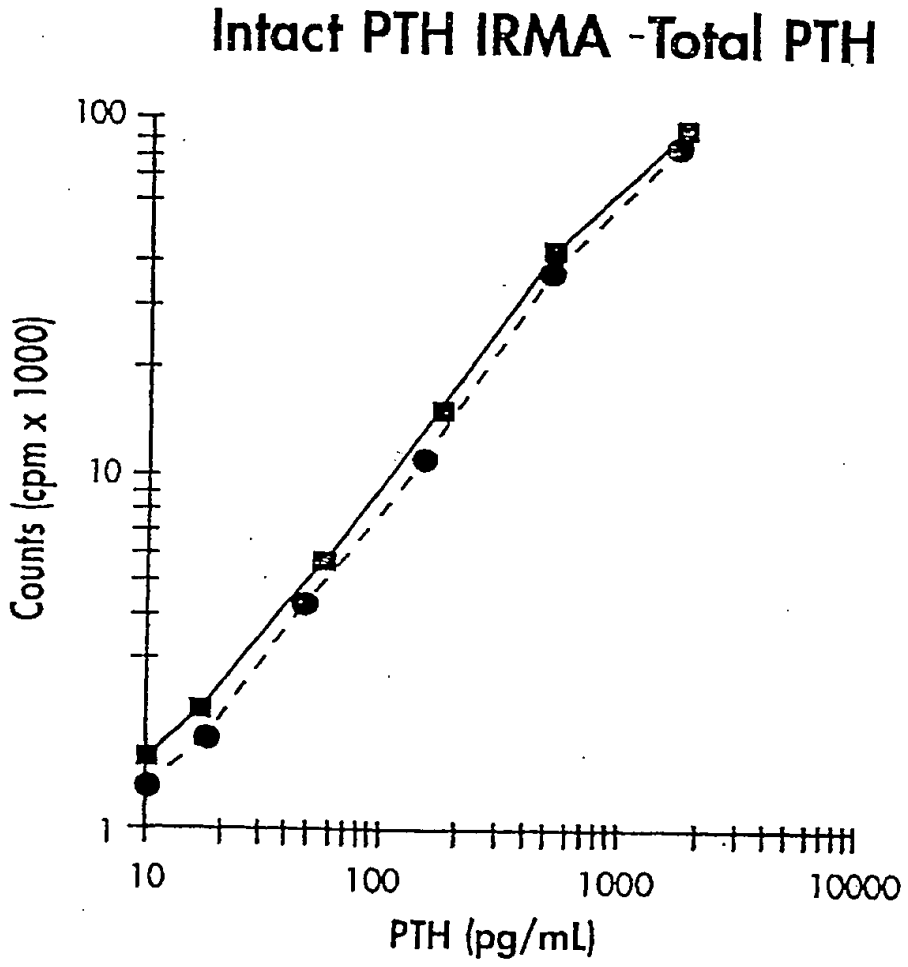

Intact PTH IRMA -Total PTH
Counts (cpm x 1000)
100
10
1
10
100
1000
10000
PTH (pg/mL)

FIG. 11

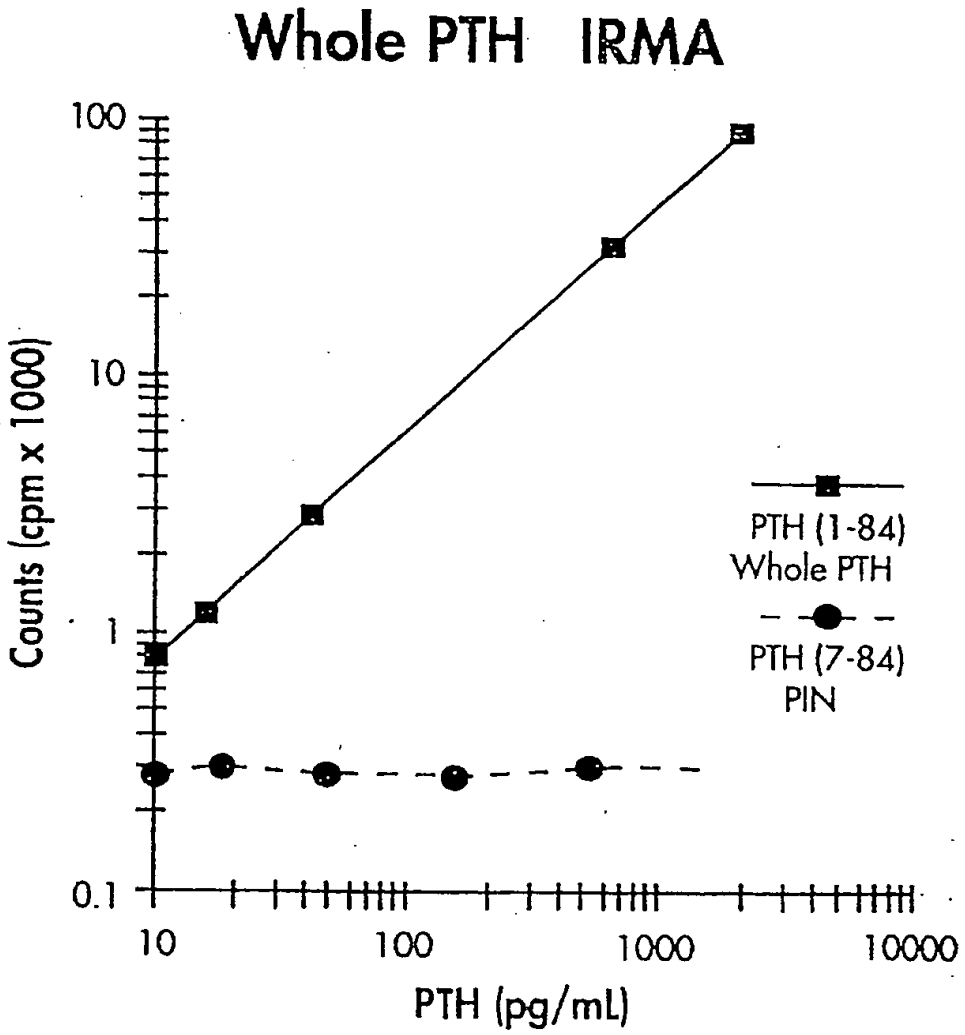

Whole PTH IRMA
100
10
1
0.1
Counts (cpm x 1000)
10
100
1000
10000
PTH (pg/mL)
PTH (1-84)
Whole PTH
PTH (7-84)
PIN

FIG. 12

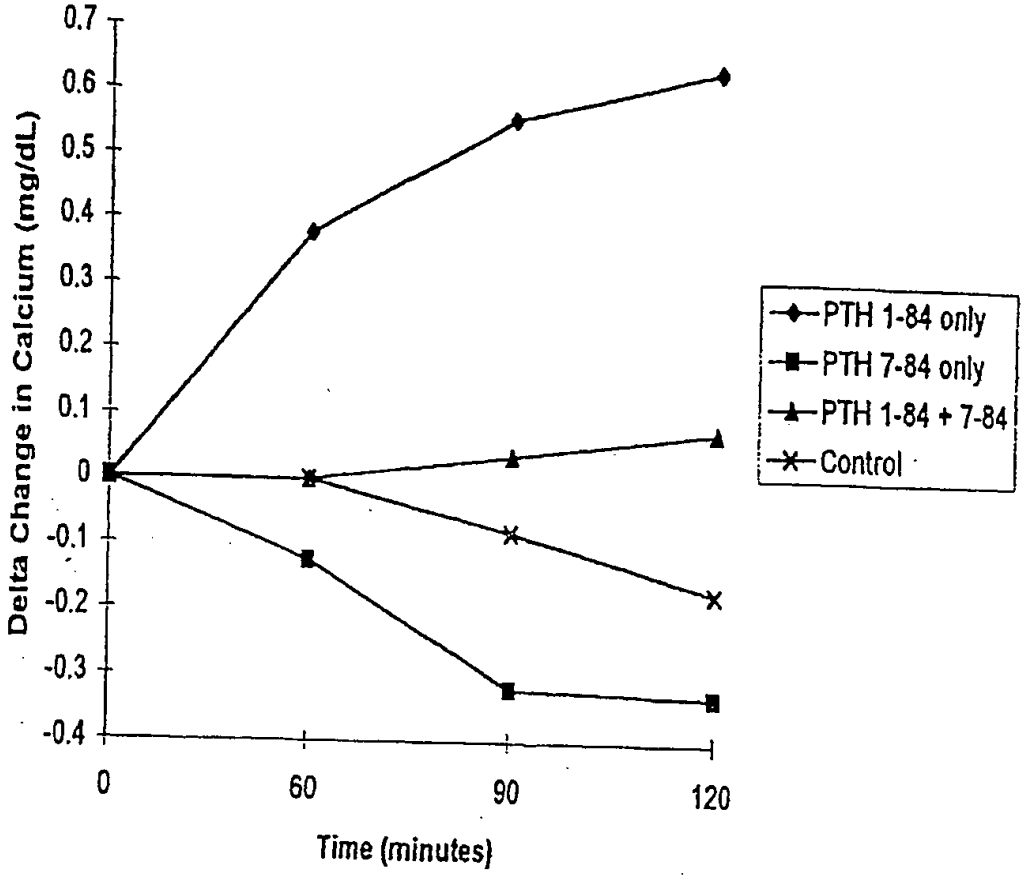

0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
-0.1
-0.2
-0.3
-0.4
Delta Change in Calcium (mg/dL)
0
60
90
120
Time (minutes)
PTH 1-84 only
PTH 7-84 only
PTH 1-84 + 7-84
Control

FIG. 13

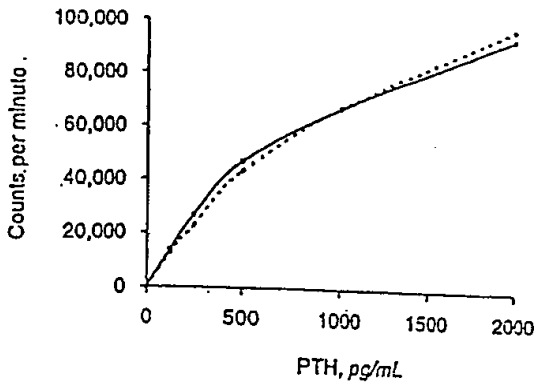

100,000
80,000
60,000
40,000
20,000
0
Counts per minute
0
500
1000
1500
2000
PTH, pg/mL

FIG. 14

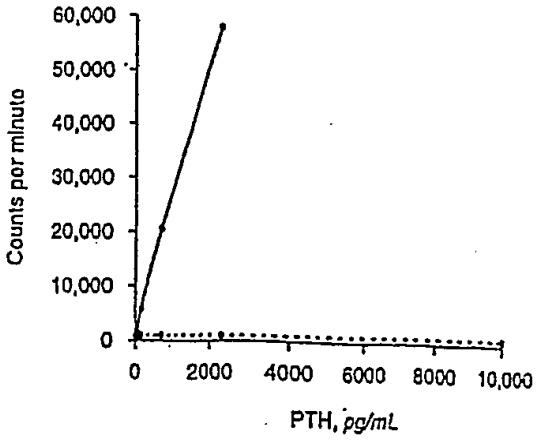

60,000
50,000
40,000
30,000
20,000
10,000
0
Counts per minute
0
2000
4000
6000
8000
10,000
PTH, pg/mL

FIG. 15

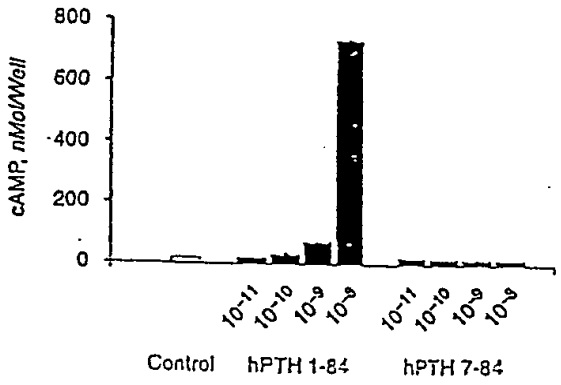

800
600
400
200
0
cAMP, nMol/Well
10-11
10-10
10-9
10-8
10-11
10-10
10-9
10-8
Control
hPTH 1-84
hPTH 7-84

FIG. 16

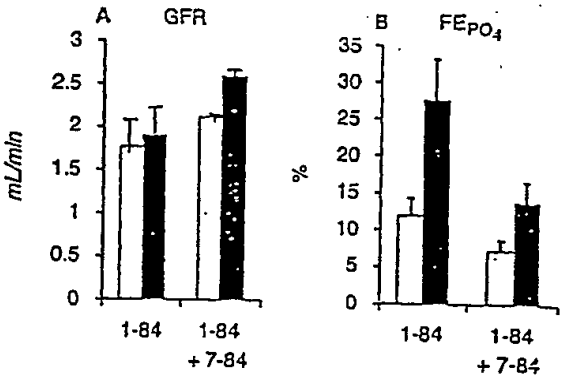

A
GFR
mL/min
3
2.5
2
1.5
1
0.5
0
1-84
1-84
+ 7-84
B
FE_{PO_4}
%
35
30
25
20
15
10
5
0
1-84
1-84
+ 7-84

FIG. 17

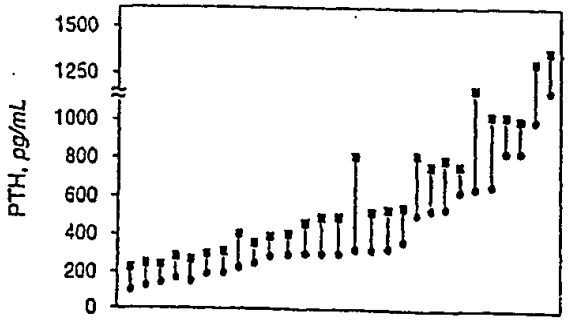

PTH, pg/mL
1500
1250
1000
800
600
400
200
0

FIG. 18

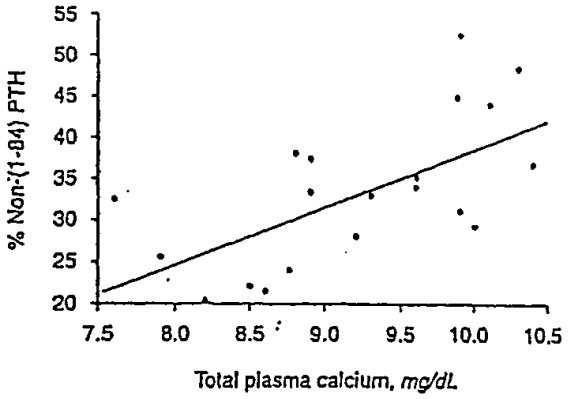

% Non-(1-84) PTH
55
50
45
40
35
30
25
20
7.5
8.0
8.5
9.0
9.5
10.0
10.5
Total plasma calcium, mg/dL

FIG. 19

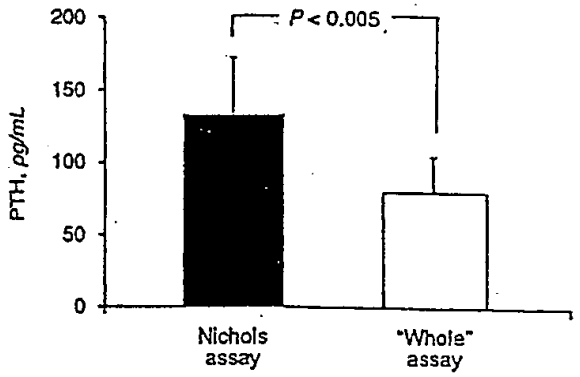
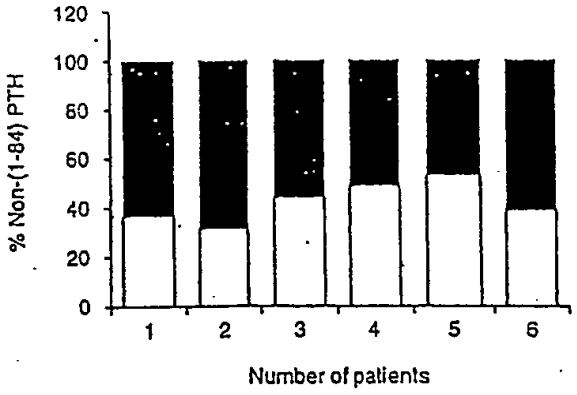

P < 0.005
200
150
100
50
0
PTH, pg/mL
Nichols assay
"Whole" assay

FIG. 20


120
100
60
60
40
20
0
% Non-(1-84) PTH
1
2
3
4
5
6
Number of patients

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